The problem of fouling in crossflow microfiltration

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THE PROBLEM OF FOULING

IN CROSSFLOW MICROFILTRATION

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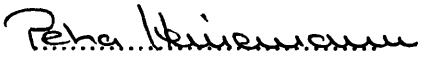

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I N M E M O R Y O F *GESCHE*

AND TO MY PARENTS

FOR THEIR SUPPORT

OF MY FURTHER EDUCATION

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SUMMARY

A serious problem in cross-flow microfiltration is membrane fouling by proteins. It accounts for loss of flux and increased rejection of solute. Previous work has indicated that flux and protein transmission are affected by, amongst other factors, the environmental conditions such as pH and ionic strength. The present experiments were designed to investigate this further.

A 0.2 μm polysulphone hollow fibre cartridge and a thin channel flow cell fitted with sheets of cellulose acetate, nylon and polytetrafluoroethylene were used to filter proteinaceous solutions in the cross-flow mode. Flux and permeate protein concentration were continuously monitored and recorded on disc using computerised monitoring. Tap water treated by reverse osmosis was first used. It was shown that water flux depends strongly on the ionic strength and pH. The addition of salt to the water increased flux by 50-75% according to the type of membrane used. Flux was highest at a low pH. Both observations were attributed to the effects of the variations on the Gouy-Chapman diffuse double layer in the pores.

Solutions of BSA, ovalbumin, whey concentrate and BiPro^{TM} gave generally a higher flux, at a pH away from the isoelectric point (I.E.P). Adding salt resulted in a flux depression, except when whey proteins were at a high concentration. In this case flux was almost doubled. The proteins of the fouling layer probably change configuration with pH influencing flux. Proteins are most compact at the I.E.P and are likely to form a densely packed layer exhibiting a high resistance to flow. This may also apply in the presence of salt. Charged proteins as in cases away from the I.E.P may form a more porous layer. Salt-protein mixtures are also likely to cause pore plugging.

BSA, ovalbumin and whey concentrate showed a maximum protein transmission around the I.E.P. At a pH different from that more proteins were rejected. This was attributed to interactions of the charged proteins with the charged diffusive layer. In contrast to whey concentrate, reconstituted spray dried BiPro^{TM} transmitted at the lowest rate around the I.E.P. Casein too showed a minimum transmission around the I.E.P. Presumably in both cases precipitation of agglomerates occurred.

The transmission of pepsin was constant at pH 3-8. Pepsin with an I.E.P > 2 was always negatively charged and transmission was thus unaffected by pH.

Adding salt to the protein solutions increased the protein transmission except at the I.E.P. A high ionic concentration leads to a compaction of the diffuse layer which presumably enlarges the permeable area, hence protein transmission.

Furthermore, it was investigated whether a low pressure would improve the protein transmission through a microporous membrane. The results demonstrated clearly that transmission increases as pressure decreases.

Flat sheets of cellulose acetate and nylon with a pore size of 0.2,0.45 and 0.6μm were used to concentrate fermentation broths. The sheets were mounted in a thin channel flow cell. The highest flux was obtained with membranes of 0.45μm which was attributed to the favourable ratio of particle size to pore size.Both membranes were used to separate intracellular enzyme from cell debris. 100% enzyme transmission was obtained for membranes of 0.45 μm and a low transmembrane pressure.

Yeast debris removal was performed with cellulose acetate and PTFE flat sheets of 0.45 μm. The cellulose acetate membrane yielded 100% enzyme transmission for a low operating pressure.

The ability of sodium hydroxide,hydrogene peroxide,sodium perborate,protamine sulfate and Terg-A-Zyme in restoring membrane properties was examined. Sodium hydroxide and Terg-A-Zyme recovered 50% of the original flux and achieved the best cleaning effect. Repeated cleaning restored this value but the initial membrane performance was never regained.

A mathematical model was designed to describe the long-term flux decline. The model parameter representing a rate constant for the protein layer and the apparent orders of reaction at which the fouling process takes place were determined for different proteinaceous systems at various operating conditions. The theoretical model fitted the experimental data best in cases of protein separation performed at the I.E.P and at all concentrations. This was attributed to the presumably weaker charge effects existing under these conditions.

CHAPTER I

INTRODUCTION

1. HISTORY AND APPLICATION

Although the first mentioned process now known as ultrafiltration was carried out by Schmidt (1856), who filtered protein and gum arabic through an animal membrane, it was during the last thirty-five years that pressure driven membrane processes have experienced a dramatic growth. The beginning of extensive commercial and laboratory use of membrane filters in the 1950s may be traced back to two major events. It was after the Second World War when the Biological Department of the US Army Chemical Corps became interested in the application of membrane filters for bacteriological analysis of water supplies. They recognised the need for rapid detection of bacteria - particularly as an early warning of micro-organisms in bacteriological warfare. Subsequently, they awarded research contracts to develop and improve existing membranes but because of bacteriological warfare concerns at this stage ,membrane and application development was classified as secret.

Another milestone was the discovery of reverse osmosis - a pressure in excess of the osmotic pressure could be used to separate water by passage through a semipermeable membrane. It was again government funding which accelerated the commercial development of reverse osmosis. The American Office of Water, Research and Technology foresaw the coming shortage in fresh water supplies and set out to develop inexpensive methods for desalinating sea water and brackish water.

Scientists had long searched for a synthetic membrane to duplicate the diffusion processes accomplished in nature when ,in 1846, Schoenbaum accidentally synthesised nitrocellulose (Schoenbein, 1846) and, in 1855, Fick made the first synthetic membrane out of nitrocellulose (Fick, 1855). He dipped ceramic thimbles into an ether-alcohol solution of cellulose nitrate and used the synthesised product for his dialysis experiments. Baranetzky (1872) introduced the concept of preparing membranes in sheets. He poured the collodion, as the ether-alcohol- cellulose nitrate mixture was called, onto glass plates. He noted that, when immersed in water, the solvent evaporated from the mixture forming pores in the membrane.

Until the turn of the century the "manufacturing" of membranes was neither understood nor reproducible. It was in 1907 when Bechhold, resuming Baranetsky's idea, succeeded in producing a graded series of membranes with varying permeability (Bechhold, (1907). A piece of hardened filter paper was soaked in a solution of cellulose nitrate in acetic acid and jelled by immersion in water. Bechhold found that the permeability was inversely related to the concentration of collodion.

He also succeeded first in estimating the diameter of membrane pores by measuring the air pressure required to expel water from water wet pores. Since the capillary force retaining water in the pores is inversely proportional to the pore-diameter, the maximum pore size can be calculated. The bubble point test is still used as a simple, non destructive test for integrity which can be applied just prior to filtration.

The first self-supporting flat disc membrane was cast by Biegelow and Gemberling (1907). They substituted ether-alcohol for acetic acid and poured the mixture onto a glass plate. The pore size was regulated by controlling the evaporation of solvents during the drying stage.

It was this process which led, in 1918, to the first production of nitrocellulose and cellulose-ester membranes on a semi-commercial scale. Zsigmondy and Bachmann (1918) cast a mixture of cellulose derivatives, solvent and water on a glass plate and regulated evaporation of the solvent by passing air in known quantitites slowly over the mixture. They also controlled humidity and temperature.

In 1927 the Sartorius-Werke company refined the Zsigmondy-Bachmann process and began commercial production of membrane filters on a small scale, but still mainly for research purposes.

The use of membranes in bacteriological analysis of water in the beginning of the 1950s, and about the same time in desalinating seawater and brackish water, led to the breakthrough in membrane use and as new applications began to emerge, the need for membranes with improved chemical resistance and heat stability resulted in investigations of other materials and methods of fabrication.

In 1960 Loeb and Sourirajan presented a new cellulose acetate membrane which possessed a salt rejection of 99% but a 200 times higher water flux than cellulose acetate membranes cast in the traditional way (Loeb and Sourirajan, 1960). The key was the asymmetry of the Loeb-Sourirajan membrane. Not the total membrane thickness but the ultra thin top layer determined the permeability. The membrane, made of cellulose acetate, acetone, water and perchlorate was cast at 0 °C and finally heated in water at 80-90 °C.

A tremendous effort was made to find materials which were more stable at higher temperature and also solvent, base and acid resistant. Regenerated cellulose - the cellulose is dissolved and then reprecipitated as a porous membrane - can be used to filter almost any organic solvent. Polyvinylchloride membranes showed improved resistance to acids and bases as well as greater strength and flexibility. Nylon also offered improved physical characteristics and solvent resistance with the exception of ethanol and methanol.

Until the early 1960s membranes were mainly fabricated by solvent-casting and still nowadays most of the commercially available membranes are manufactured by this type of phase inversion. A polymer-solvent solution is cast on a suitable support and immersed in a non solvent coagulation bath. The solvent is exchanged by the non solvent and the polymer precipitates forming a porous structure. Depending on the ratio of polymer, solvent and non-solvent, ultra and microfine pore sizes are obtained.

In 1963 it was discovered that thin plastic films could be track-etched to produce microporous membranes. The film was exposed to a beam of fission fragments. The atomic particles penetrated the material and weakened the chemical bondings. A subsequent etching bath created a precise cylindrical pore around the irradiated centre whose size was controlled by the duration of the etching phase. The most distinguishing feature of the track-etched membranes, better known under the trade name Nuclepore(TM), exhibits the extreme uniformity of the pore structure and the smooth surface (Nuclepore Bulletin, 1983). Nuclepore(TM) membranes are available in polycarbonate and polyester.

Another class of polymeric membranes made from inert plastics such as polypropylene, polyvinylidene fluoride and polytetrafluoroethylene was launched in the early 1970s. The membranes are manifactured by exposing non-porous films to uniaxial and biaxial stretching hence inducing a series of permanent microtears (Druin et al, 1974). The

advantage of the polytetrafluoroethylene membrane is its exceptional chemical inertness and thermal stability. In the mid 1970s a uniform porous polypropylene tube was formed by a thermal inversion process. Polypropylene was dissolved in an organic solvent at elevated temperatures. The polymeric solution was then cooled until the polypropylene precipitated out around the remaining solvent (Mulder, 1986).

Lately inorganic membranes have gained more and more interest, chiefly because of their mechanical and chemical durability. They are made of sintered metals, ceramics, stainless steel and glass fibres.

Prior to the 1960s the principal geometrical configurations of available membranes were flat sheet structures or tubular configurations. It was in the early 1960s when the spiral-wound module was invented (Westmoreland, 1968; Bray, 1968) utilising sheet-stock membranes. The spiral wound module is one of the most successful designs on the RO market but is also applied in ultra- and microfiltration devices. Demand for a high area to volume ratio device resulted in the development of the hollow-fibre configuration. In the mid 1960s the first attempts were made to manufacture cellulose triacetate and aromatic polyamide hollow fibres. Although both fibres had water fluxes considerably lower than conventional cellulose acetate membranes they had gained a considerable market share already at that stage because of the ease and low cost of spinning. Extremely large areas could be produced at low cost. Hollow fibre systems offer the advantage that fluids are filtered by radial outward flow at low pressure without risk of membrane rupture. They found therefore, applications in the low pressure, ultra- and microfiltration processes. A supplement is that hollow fibre devices can be operated in a reverse flow mode. Foulants are dislodged and an acceptably high overall transmembrane flow rate can be maintained.

A key concept in membrane processes is concentration polarisation. Macromolecules which are rejected at the membrane surface build up their concentration at the membrane surface until the rate at which they are convected back into the bulk liquid by eddy diffusion is equal to the rate at which they are brought to the surface by the transmembrane flow. When the membrane surface concentration reaches a particular value, a limiting flux occurs, which is not exceeded when transmembrane pressure is raised,but only when the mass transfer coefficient is increased by increasing the cross-flow velocity, or more precisely, the Reynolds number. Controlling the hydrodynamic conditions was the key concept leading to the development of high-capacity leaf modules

produced by Dorr–Oliver in the mid 1960s. They consisted of closely spaced, parallel, membrane bearing plates allowing the feed to flow under turbulent conditions. Amicon introduced thin channel flow systems whilst Abcor launched a spirale module – a cellulose acetate membrane was casted on the inside of a porous polyethylene tube.

More recently, plate and frame stack systems have been produced. Amicon and Millipore market small–scale units, but DDS has been very successful in marketing large–scale plate and frame modules for ultrafiltration. Plate and frame systems are more expensive than other systems but they offer the advantage of separate permeate lines at each plate and the ability to interchange membrane media which cannot be cleaned or have ruptured.

A new technical approach was made by the Swiss company Sulzer. They engineered a rotating pressure filtration laboratory unit. The suspension or solution to be filtered is pumped continuously into the pressurised annular gap between the rotating inner filter cylinder and the outer container wall. The ensuing Taylor vortices flow across the filter surfaces of the inner and outer cylinders. The concentrate is withdrawn from the annulus by a pump which maintains the pressure in the filter chamber. The filtrate is removed from the core (Sulzer Bulletin, 1985).

A list of some manufacturers of membranes and filter modules is given below in Table 1.

TABLE 1 Manufacturers of Cross-Flow Filter Membranes and Filter Modules

Manufacturer	Design	Membrane	Pore size range
Amicon, USA	Hollow fibre cartridges	Polysulphone	30,000-300,000 daltons
	Plate and frame devices		0.45 & 0.6 μm
A/G Technology, USA	Hollow fibre cartridges	Polysulphone	5,000-50,000 daltons 0.1-0.45 μm
APV, UK	Multi-tubular devices	Sintered ceramic (Aluminium oxide)	0.4-10 μm
Dominic Hunter, UK	Flat sheets Filter cartridges	Cellulose acetate Nylon Polypropylene PTFE	0.1-3 μm 0.2, 0.45, 0.65 μm 0.6-100 μm 0.2
Enka, The Netherlands	Tubular devices	Polypropylene	0.2 μm
Gore	Flat sheets	PTFE	0.02-10 μm

Koch (Abcor), USA	Flat sheets Spiral wound device	Polymers	0.2-2 μm
Millipore, USA	Stack systems	PTFE PVDF Cellulose	0.2-3 μm
Nuclepore, USA	Spiral wound device	Cellulose Polysulphone	
	Flat sheets	Polycarbonate Cellulose	0.01-12 μm 0.1-5 μm
	Filter cartridges	Polycarbonate Polyester	0.1-1 μm
Paterson Candy, UK	Tubular devices	Cellulose acetate Polysulphone PVDF	10^3 daltons 10^4 daltons 10^6 daltons
Wafilin, The Netherlands	Tubular devices	Polymers	

The distinction between reverse osmosis, ultra- and microfiltration is quite arbitrary since they operate on the same basic principles. The former is a high pressure process often operating over 100 bar. RO membranes tend to have no pores but rather an amorphous region, UF membranes have distinct pores whilst MF membranes, with the exception of the track-etched membranes, tend to be fibrous. A widely quoted definition based on the size of the molecules or particles retained by the membranes was suggested by Porter and Michaels (1971).

Reverse Osmosis (RO)	:	0.0001 to 0.001 μm
Ultrafiltration (UF)	:	0.001 to 0.1 μm
Microfiltration (MF)	:	0.1 to 10 μm

The above definition is useful as a guide to select the appropriate membrane for a particular application. MF membranes can be used to retain particulates, micro organisms, viruses and colloids, as well as to separate particles from products with a size substantially smaller than the pore size. UF membranes on the other hand, are commonly applied in concentrating macromolecules such as proteins or organic compounds of molecular weight over 1000 MW in solution. RO membranes are capable of rejecting ionic species such as sodium or chloride with sizes of the same order of magnitude as the water molecule.

Lists on the application of ultrafiltration membranes are given by Bailey (1977), Michaels (1981), Tutunjian (1983) and Bell (1985). One of the earliest and most important use of UF has been in the recovery of electrocoat paint primer. Prime coating of automobiles and frames was readily appreciated by industry but the reliable and economic recovery of colloidally dispersed paint from the rinsings as well as the removal of soluble impurities and corrosion products from the electrocoating baths by means of ultrafiltration, was the definite breakthrough of the electrocoat process.

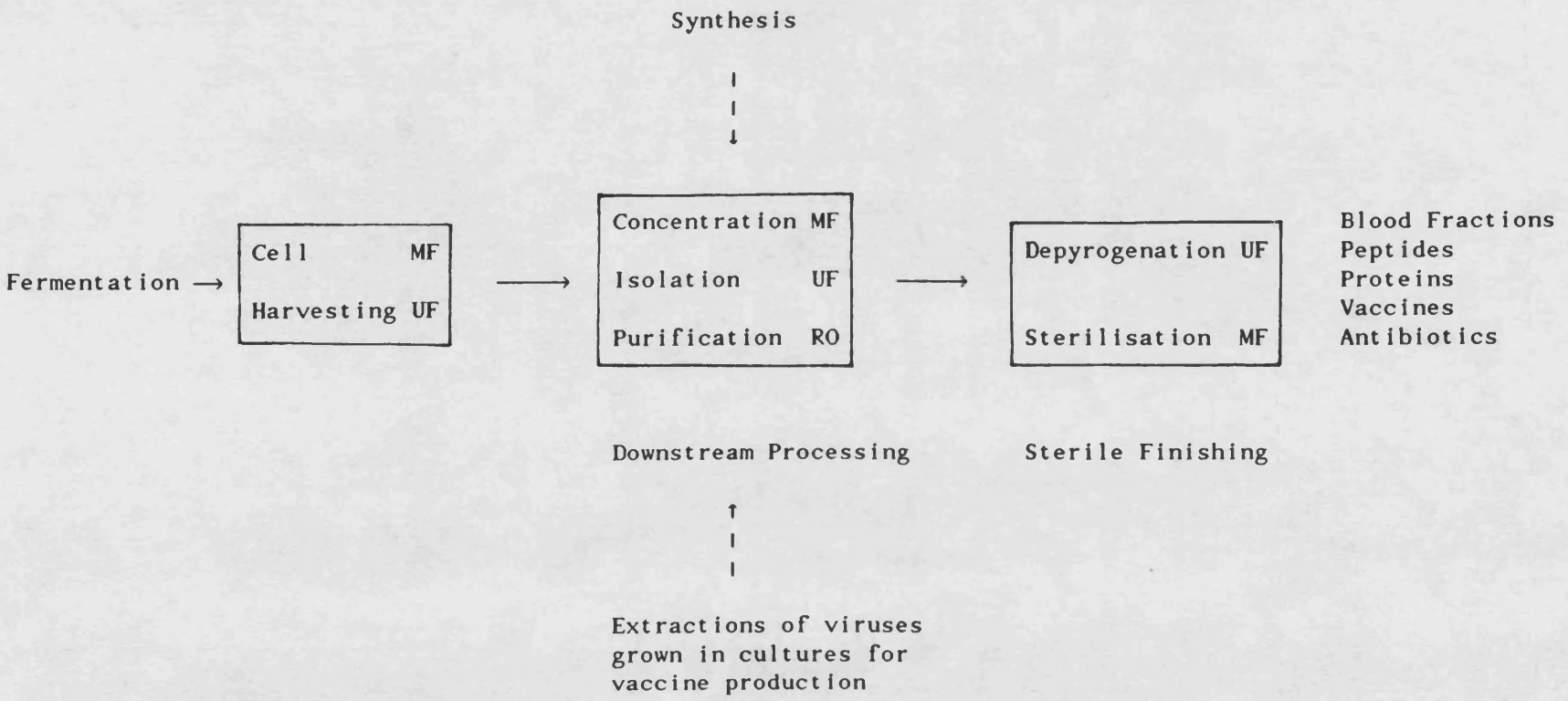
Among the earliest potential applications of UF in the food and dairy industry has been the recovery and concentration of valuable proteins from cheese whey. In the past the liquid by-product from cheese manufacture was regarded as a waste product to be disposed of as cheaply as possible. Anti-pollution legislation at the beginning of the 1970s (Delaney et al, 1972) forced the dairy industry to utilise whey. Up to that date the major processed form of whey was whey powder but its use as animal food barely covered manufacturing costs. The fact that 70% of the nutritive value of the milk remains in the whey suggested the use of whey for human nutrition, but a major limitation was the fact

that dried whey consists mainly of denatured protein. At that time membrane separation technology was already well developed. It offered the possibility to solve the whey disposal problem by concentration and to realise the concept of whey as a raw food material by fractionating it into its components. The trend to reuse cheese whey grew very quickly in the beginning and ceased because of the difficulties in selling the raw food material.

A growing area of processing milk by reverse osmosis and ultrafiltration is the manufacturing of yoghurts and cheese with limited whey by-production. Reverse Osmosis and Ultrafiltration plants producing 'soft cheeses' such as Feta, Camembert and Mozzarella are operating in France and the United States. Hard Cheddar cheese is made in Australia. The application of Reverse Osmosis and Ultrafiltration systems gave, in general, a higher yield compared to the traditional manufacturing process (Phillips, 1987).

Another important industrial application of membrane systems is the purification and concentration of biochemicals such as vaccines, peptide hormones, antibiotics and plasma proteins. The success of recombinant DNA science in manipulating micro-organisms to express mammalian genes has made it possible to produce a larger number of pharmaceuticals and biologicals through the fermentation route. The bottleneck is still the isolation and purification. Ultra- and microfiltration are important processes in downstream processing systems with a minimal loss of labile products due to denaturation or decomposition. Fig.1a shows how membrane technology can be incorporated in processing biochemicals.

Fig.1a Industrial Production of Pharmaceuticals and Biologicals



Traditional cell harvesting methods like settling and dead end filtration are tedious, time-consuming and do not always achieve the desired concentration. Centrifugation is likely to disintegrate the product, may generate aerosols and is usually less cost effective.

Henry and Allred (1972) concentrated Micrococcus sp. by using a microporous filter in the dead-end mode with line vacuum and obtained a filtration rate of 0.5 $\ell/m^2/h$ compared to an average permeate flux of 100$\ell/m^2/h$ when the same filter was used in the cross-flow mode. Schutte et al (1983) investigated different methods to concentrate a Bacillus cereus culture. The self-flocculating strain was left to settle. The two-fold concentration which was achieved within ten hours could not be exceeded by allowing more settling time. The desired concentration factor of three was obtained with both cross-flow filtration and centrifugation by means of a nozzle separator.

Hanisch et al (1982) also came to the conclusion that cell harvesting with a hollow fibre system is more convenient and economical than centrifugation. Le et al (1984b) compared the running costs of crossflow microfiltration and centrifugation. They concentrated Erwinia carotovora, a micro-organism which produces α-asparaginase a therapeutic enzyme which treats some forms of leukaemia. The cost of the MF system was found to be 30 - 50% less expensive than centrifugation. The relative cost comparison breakdown is given in Table 2.

Microbial harvesting has also been described by others (Tanny et al, 1980; Kroner et al, 1984) and was first carried out on a large scale in 1976. Reid and Adlam (1974) concentrated 60ℓ of Corynebacterium parvum in a glucose broth to 8ℓ within 3.5 hours by using two membrane filter stacks with a total filtration area of 5100 cm^2. Zahka and Leahy (1985) concentrated 400 litres of Escherichia coli broth through 2.3 m^2 filtration area by a factor of 20 and an average flux of 26 $\ell/m^2/h$. They also processed 250 litres of Mycoplasma sp. through 4.5 m^2 filtration area and achieved a 50 fold concentration at an average flux of 8.9 $\ell/m^2/h$.

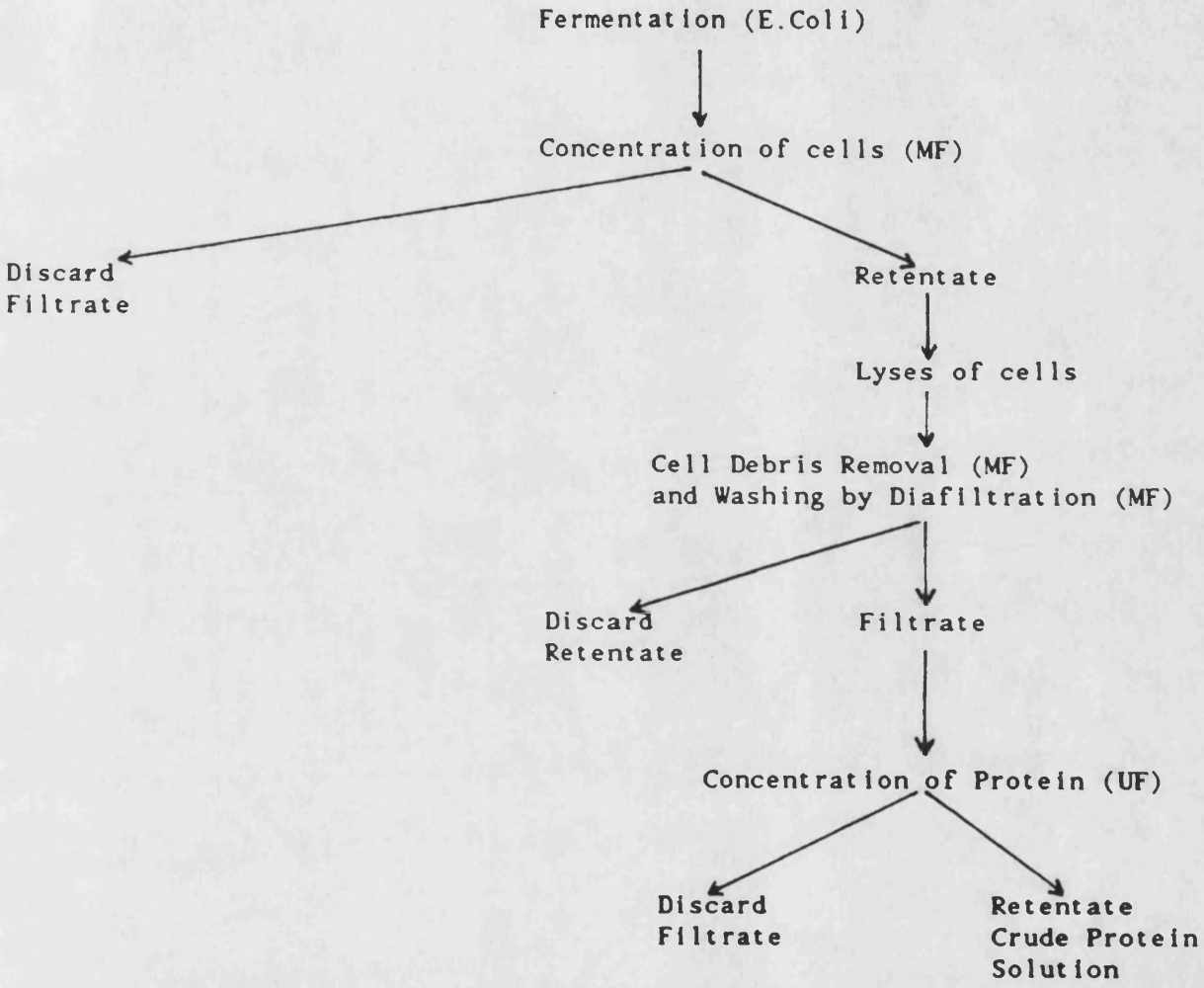
Microfiltration technology also gave hope that one of the most difficult liquid/solid separations, cell debris removal, could become more convenient. Griffith et al (1979) separated a highly viscous polymer from the biomass of its parents fungus. Quirk and Woodrow (1983), Le et al (1984a) and Datar (1985) investigated intensively enzyme separation from cell debris. They concluded that microfiltration is suitable to tackle this difficult downstream step. Gabler and Ryan (1985) demonstrated the joint application of

TABLE 2 Cell Harvesting Running Costs (Ref.see Scott,1986): Comparison Between Centrifugation Microfiltration (Calculated as a Percentage of the Total Centrifugation Costs)

Process Cost	Centrifugation Batch size/ℓ 1-300	Centrifugation Batch size/ℓ 5000	Microfiltration Batch size/ℓ 1-300	Microfiltration Batch size/ℓ 5000
Membrane Replacement	-	-	11.0	33.3
Energy	4.6	5.6	0.6	1.1
Labour	36.3	33.3	18.3	6.7
Depreciation	30.5	33.3	8.5	17.8
Overheads	10.0	10.0	4.9	6.9
Annual Maintenance	18.3	17.8	3.7	3.3
	100	100	47	69.1

MF and UF to recover the intracellular human Immunoglobulin G from E. coli broth. The flow chart of all processing steps is shown in Fig.1b.

Fig.1b Recovery of Human Ig G from Escherichia Coli Broth



Although the applications of UF and MF in the pharmaceutical and food processing industries seem to be most attractive, their full integration has been delayed because of two major drawbacks. Firstly the fact that proteins are particularly susceptible to adsorption onto surfaces. They tend to foul membranes and by doing so change the separation and transmembrane flowrate characteristics. The rejection of proteins is a major problem in microfiltration, since one of the main applications is separation. The product is very often the filtrate rather than the retentate which means loss of product

because of rejection. A possibility to recover the retentate is diafiltration but this is an additional step in downstream processing requiring very often a consequent concentration and increases, therefore, the total cost of the product.

The second reason is that plants operating in the dairy industry are subject to hygenic requirements. Regular cleaning and sanitizing operations can be quite destructive for membranes depending on the applied chemicals. Frequent renewing of membranes raises the total cost. However, UF and MF have also reduced the total cost in some pharmaceutical processes despite the frequent replacing of membranes (Cook, private communication).

2. THEORETICAL BACKGROUND

Early models of cross-flow membrane fouling were on the cake filtration theory with convective deposition modified by mass transfer away from the membrane surface. More recently it has been recognised that fouling is not a single phenomenon but rather a series of different events, often occurring sequentially in time on the same system, and also differing in some essential features from system to system. There is still no one generally accepted theory of fouling, although there are some aspects about which there is some measure of agreement.

The first factor that is dominant in filtration processes is the effect of concentration polarisation. Macromolecules accumulate at the membrane surface and build up into a layer until a limiting concentration is reached. It is then asumed, described by the gel polarisation theory, that a gel starts to form and all further build-up of solute must occur by thickening of the gel layer at the membrane surface. This leads to an increased resistance which reduces the transmembrane flux until the convective mass transfer of macrosolute to the membrane surface, exactly counterbalances the diffusive transport back to the bulk liquid. The corresponding steady state transmembrane flux can be altered only by changing the net rate of back-diffusion, or in other words, by varying the mass transfer coefficient which depends on the local Reynolds number and the Schmidt number.

This gel polarisation model was first presented by Michaels (1968) and more intensively studied by Blatt et al (1970). There is quantitative agreement between this model and experimental data for the change of flux with pressure, bulk concentration and mass

transfer coefficient at the steady state of the short term fouling for a given system, but it breaks down in cases where the system changes, when dynamics are considered and it also does not hold for long-term fouling. Porter (1972) discussed the differences that exist between the gel-polarisation theory and the experimental data. He explained the differences with the so called tubular pinch effect. Kozinski and Lightfoot (1971) investigated the effect of viscosity and diffusivity variations on concentration polarisation during ultrafiltration of protein solutions. They reported that these effects are very small and insensitive to the forms of concentration dependence postulated. However, they extended their studies to develop a model for a rotating disc membrane taking into consideration concentration dependent diffusivity and viscosity (Kozinski and Lightfoot, 1972).

The agreement of the theory with the experimental data was reasonable, although they observed some hysteresis in the permeation rate at low ionic strength. They attributed this effect to instability of proteins at low ionic strengths and their tendency to undergo polymerisation. This hysteresis was later recognised to be a consequence of fouling.

Shen and Probstein (1977) attributed the difference between theory and experiments to variable transport properties of the macromolecular solution. Their results led to an agreement with Kozinski and Lightfoot (1971) that the concentration dependence of viscosity has little effect on the limiting flux but the diffusivity dependency of the concentration could not be ignored. Trettin and Doshi (1980) suggested that diffusivity of BSA was independent of the concentration by showing that a constant property integral method (assuming a constant diffusivity) yielded less than 3% error between the theoretical prediction and experimental data for BSA. However, since viscosity of lactalbumin for example, has been shown to be concentration dependent, Trettin and Doshi's results may be the result of concentration effects fortunately cancelling out.

Probstein et al (1978) developed a semiempirical formula for the limiting flux which considered the diffusion coefficient evaluated at the gelling concentration. In order to explain the permeate-flux-pressure behaviour another model was successfully applied. Goldsmith (1971), Kozinski and Lightfoot (1972), and Leung and Probstein (1979) showed that the osmotic pressure is an important factor in the ultrafiltration model. To what extent the osmotic pressure model is valid depends mainly on the molecular weight of the solutes. It is doubtful if it can be applied in the case of macrosolutes like proteins or cells. A limiting concentration for polarisation appears to occur. It is probably not a gel

concentration since:

1. The limiting concentration is not a constant for the solute, but varies by as much as 50% with different membrane types (Porter, 1972; Nakao et al 1979).

2. The experimentally determined limiting concentrations are often within the solubility limits of the solute (Goldsmith, 1971).

3. There is a slow decline of permeate flux with time.

4. Permeability loss is not restored by chemical cleaning.

Since the gel-polarisation theory leaves many questions unanswered there has been a move to other models in the past few years. Howell and Velicangil (1982) proposed a three-stage model. According to this model the concentration polarisation process is completed within the first few seconds, followed by an adsorption step in which the proteins adsorb to the membrane causing flux decline. This process occurs during the first 15 minutes. Further flux loss is attributed to a polymerisation or gelation mechanism in which more protein from the bulk solution reacts with the adsorbed proteins. The advantage of this model is that it does not consider the pressure independent flux which is still the key issue in filtration processes.

Fane et al (1980) described the relation of concentration and flux in terms of the total observed resistance which is the sum of the resistances offered by the membrane, the dissolved solids and the suspended solids. They observed that this "Resistance in Series" model tends to underestimate the flux. Fane et al (1980) attributed the discrepancy to the reduced influence of the dissolved solids on the total resistance in the presence of suspended solids. Furthermore, they claimed that long-term flux decline is not determined by pore plugging but can be ascribed to an ageing process in the gel layer. Le and Howell (1984) offered an alternative model to describe the limiting flux assuming that the membrane pores can be either unblocked, partially blocked or fully blocked. They claim that in the pressure independent region a further increase in pressure will cause a transient increase in flux. The proportion of blocked pores will increase, hence reducing the flux to its limiting value. Vice versa, if the pressure is reduced the flux shows a transient decrease resulting in an increase in the proportion of unblocked pores, hence the flux increases to its limiting value.

The limiting flux model allows semi-empirical modelling of ultrafiltration performance but it does not allow prediction of the slower rate of flux decline occurring after the initial polarisation has finished. Neither does it allow prediction of the influence of the fouling layer on the rejection characteristics of the membrane - an important factor when solute fractionation is required.

(Aimar and Sanchez, 1985) derived a model which takes into account the osmotic pressure and the concentration dependency of the viscosity. The limiting flux depends on the geometry, hydrodynamics and concentration but does not require the assumption of a gelling concentration. They claimed that the variation of the viscosity in the boundary layer has a fundamental importance and that it is this phenomenon, superimposed on the osmotic pressure, which causes the existence of a limiting filtration flux.

Table 3 shows a summary of the above mentioned theoretical models.

TABLE 3 Theoretical Models Describing the Flux Decay in Cross-Flow Filtration

Description	Models			Reference
	$JC = D\frac{dC}{dx}$		$Sh = \frac{k_m d}{D}$	Michaels (1968)
Concentration polarisation model	$J = k_m \ell n \frac{C_s}{C_b}$	pre-gel region	$Sh = f\left[Re, Sc, \frac{D}{L}\right]$	
	$J = k_m \ell n \frac{C^*}{C_b}$	gel-polarised region	$C^* = C_g$	
	The concentration polarisation model relates flux decline to the formation of a polarised layer. Flux becomes limited when the layer turns into a gel.			
	$JC = D\frac{dC}{dx}$			
	$Sh = A\, Re^{0.5} Sc^{0.33}$			Kozinski and Lightfoot (1971)
	$A = 1.0\left[\frac{C_b}{C_s}\right]^{-0.355}$	μ,D const.		
	$A = 0.76\left[\frac{C_b}{C_s}\right]^{-0.355}$	μ var., D const.		

$$A = 1.0 \left[\frac{C_b}{C_s} \right]^{-0.41}$$

μ const., D var.

Concentration polarisation model considering a concentration dependent viscosity and diffusivity

---

Osmotic pressure model	$J_p = K\Delta P$ $J = \frac{\Delta P - \Delta\pi}{\mu(R_m+R_A)}$	pure water flux flux of a macrosolute solution	Goldsmith (1971)

The osmotic pressure model relates flux decline to an increasing osmotic pressure at the membrane surface reducing the transmembrane pressure

---

Resistances in series - model	$J(t) = \frac{\Delta P}{\mu \sum R_i}$	$R_i = R_m+R_{b\ell}+R_A+R_g$ $R_i = f(t)$	Fane et al (1980)

The resistance in series model relates flux decline to particle adsorption, convective deposition and ageing of the deposit layer

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Limiting flux model	$J = \frac{J^2}{J^*}\frac{C_s}{C^*} + \left[1 - \frac{C_s}{C^*}\right] J_p$	pre-limiting region	$J_p = \frac{\Delta P}{\mu R_m}$	Le and Howell (1984)

The limiting flux model relates the limiting concentration at the surface to particle adsorption

---

Model describing the growth rate of the deposit layer	$\frac{d\ell}{dt} = k_R C_b^2 \exp\left[\frac{2\Delta P}{k_m \mu (R_m + R_A)}\right]$	$R_A = \frac{\ell}{P_g}$ $\frac{d\ell}{dt} = k_R C^2 s$ $C_s = C_b \exp\left[\frac{J}{k_m}\right]$ $Pg = \frac{d^2}{180} \frac{\bar{\epsilon}^{-3}}{(1-\bar{\epsilon})^2}$	Howell and Velicangil (1982)
	The model calculates the growth of the deposit layer and assumes particle adsorption		
Osmotic pressure model considering a concentration dependent viscosity	$\Delta P = (R_m + R_A)J + \sum_{i=1}^{3} a_i C_s^i$ $J = k_m \ell n(C_s/C_b)$ $J^* = A v^y C_b^{-X}/e^X$	$\Delta\pi = \sum_{i=1}^{3} a_i C_s^i$ $k_m = A\, v^y\, C_s^{-X}$ $X = a(x-y)$ A,a,y,x, = constants	Aimar and Sanchez (1985)
	The limiting flux depends on the variation of viscosity with concentration		

3. FOULING AND TREATMENT OF MICROPOROUS MEMBRANES

Whereas concentration polarisation is a well-understood phenomenon the more serious problem in UF and MF is membrane fouling. It accounts for severe losses in hydraulic permeability and affects the rejection properties of the membrane. It has been established that fouling is not only a single step but a multistep process:

- Plugging of pores
- Adsorption within the pores
- Adsorption on the membrane surface
- Long-term fouling

Moderately sized particles are convected into the membrane and plug the pores. The effective free permeable area is therefore reduced.

Milsic and Aim (1986) used an electron microscope enlargement to demonstrate the close-up of a pore by proteins.

There is some evidence that flux depression is minimised with small pore size membranes for MF. Le and Howell (1985) suggested that larger pore sizes allow physical jamming whilst smaller pores do not admit any blockage by bridging the pores. Watanabe et al (1979) showed that a higher flux membrane with lower rejection suffered a larger solute deposit than a lower flux but high rejection membrane. Le et al (1984a) obtained a higher flux with an 0.45 μm membrane compared to an 0.6 μm membrane made of the same material. Le et al (1984a) suggested that particles screen the small pores but enter and plug the larger pores. Smaller particles may pass through larger pores but in at least some of the cases, solute will adsorb to the surface of the membrane and within the pores, reducing their size and increasing rejection whilst decreasing flux.

Initial pore plugging and adsorption are generally completed within ten minutes of contact between protein and membrane (Le, 1982). This can cause a more than ten-fold reduction in the membrane hydraulic permeability relative to the measured pure water permeation rate. Long-term fouling accounts for a further relatively slow, continuous decline in permeation rate which is observed over hours or days.

The fouling process has been ascribed to the formation of a slowly consolidating, gelatinuous layer on the membrane surface (Michaels, 1968) or protein polymerisation (Velicangil, 1979). In reality it is not possible to distinguish between the various components; some may overlap (Fane, 1983) and some may be present in the absence of others. López-Leiva and Matthiasson (1981) demonstrated with a special experimental set-up that flux depression occurred although concentration-polarisation was not present. They concluded that the increased transmembrane resistance was due to an adsorbed layer of proteins on the membrane, whilst concentration polarisation was found to take place in all pressure driven filtration processes regardless of the nature of the solutes. Fouling is strongly associated with particular solute/membrane interactions. It is well known and described in manifold literature (eg Macritchie, 1978; Lundström, 1983; Norde 1981) that proteins are in a class of substances especially susceptible to adsorption on non-biological interfaces.

Proteins are especially prone to foul nearly all membranes as they have regions of positive and negative charges, hydrophobic regions and possess reactive side groups, for instance, sulphydryl groups, which can interact to form disulphide bonds. (Le, 1982). This causes immense problems in the field of artificial organ transplantation and also, with less serious consequences, in the field of industrial membrane filtration in food and biotechnology process industries. Busby and Ingham, 1980 noted that several membranes which readily passed polyethylene glycol exhibited increased rejection of the synthetic polymer and showed reduction in flux when albumin was present. The same authors demonstrated elsewhere (Ingham et al, 1980) that, unlike concentration polarisation, fouling is irreversible. It soon became clear that membranes of hydrophobic nature are more prone to adsorb proteins compared to those of a hydrophilic nature (Matthiasson, 1983).

The adsorption of protein is also affected by pH and ionic strength but there are some inconsistencies to be found in the literature. Matthiasson (1983) found that the amount of adsorbed protein decreases when the pH value increases. Fane et al (1983a) and Zeman (1983) on the other hand, noticed a maximum of adsorption at the iso-electric pH. (I.E.P). Aimar et al (1986) confirmed the results of Matthiasson but restricted this observation to the bulk concentration. For protein concentration larger than 10 g/ℓ Aimar et al (1986) found the minimum of adsorption occurring at the I.E.P. Reports on the influence of ionic strength are even more contradictory. Whereas Fane et al (1983a) and Zeman found the adsorption to be increased in the presence of salt, Matthiasson (1984) observed the opposite. Some explanation of these discrepancies may be found in the work

done by Suki et al (1984). They clearly showed that a discussion about the influence of pH cannot neglect the type of membrane used.

Membranes made of the same material but supplied by different manufacturers gave significantly different data when subjected to the same protein solution at different pH. They assigned the difference to membrane surface characteristics such as porosity and pore size distribution. As a direct consequence of protein adsorption the hydraulic resistance of a membrane depends also on pH and ionic strength. Fane et al (1983a), Suki et al (1984) and Aimar (1986) found a maximum hydraulic resistance around the I.E.P. whereas Matthiasson reported an increasing membrane permeability with a decreasing pH value. The presence of salt increased the hydraulic resistance at high and low pH but showed little effect at the iso-electric point (Suki et al, 1984; Fane et al, 1983a).

Little work has been published on the effect of pH and ionic strength on the rejection characteristics which is more significant in the case of microporous or partial retentive membranes. Fane et al (1983b) showed that rejection is lowest at the I.E.P. and almost 100% at a high pH. A salt supplement reduced the rejection significantly. Le and Atkinson (1985) improved enzyme transmission from 20% to 75% by adding phosphate salt to 200 mM.

The problems of reduced throughput capacity, increased power consumption, varying separation characteristics and reduced membrane life associated with fouling led to an intensive search for an adequate solution.

Kopecek and Sourirajan (1969) noted significantly higher flow rates with anisotropic reverse osmosis membranes when they were used in an open side up position for distilled water filtration. Lefebvre et al (1979) reported a comprehensive application of this technique, better known as "back pressure" or "backwash" treatment, with their polyamide ultrafiltration membranes. Le and Billiet (1984) used Aspor^{TM} microfiltration membranes to separate the intracellular enzyme paracetamolase from the fermentation broth. When the membrane was placed in the tight side up configuration a more than 80% decline in enzyme transmission was observed within 40 minutes compared to no rejection when the inverse configuration was applied. The literature offers a number of methods to diminish or prevent fouling by either pretreating the feed solution or changing the properties of the membranes.

A comprehensive list of pretreatments given by Sivik and Matthiasson (1980) and completed by Matthiasson (1985) is shown in Tables 4 and 5.

TABLE 4

Pretreatment of the Feed Solution (ref. see Sivik and Matthiasson 1980)

Process	Product
Heat treatment and pH adjustment	whey
pH adjustment	whey
ion exchange	whey
Ca-sequestering agents (EDTA)	whey
Glycerol addition to feed solution de,	polypeptide, enzyme
Change of ionic strentgh	whey
Modification of side chain (sulfhydryl-, carboxyl-)	whey
Pre-ultrafiltration	whey

TABLE 5

Changing the Properties of the Membrane Surface (ref. see Matthiasson 1985)

Type of manipulation	Product
Charged membranes:	
sulphonate polymer	protein solution oily particles
sulphonation, amination electrically polarised- electric membranes	
Immobilisation of enzymes	
trypsin	sewage
proteinase P	albumin hemoglobin
papain	whey
cystein	whey
Chemical adsorption:	
non-ionic nonyl phenol polyethoxylates	BSA
lecitin	soy milk
PEG	whey
PEG	BSA
Protective cover: fixed: microfilter dynamic: hydrophilic silica	NaCl

Cleaning of membranes is performed to recover flux and rejection characteristics at least to some extent, as well as sanitising the filtration system. Acidic and alkaline cleaners or enzyme active detergents are used widely.

Disinfectants like hypochlorite or hydrogen peroxide sanitise the system efficiently (Haagensen, 1983).

Belfort (1977) and Haagensen (1983) reviewed cleaning and sanitising techniques for membrane systems.

4. Objectives of the Present Investigation

The future expansion of cross-flow microfiltration is mainly dependent on an effective and reliable fouling treatment. In order to develop an appropriate method a better understanding of the fouling process and the factors determining it is required. One objective of the work presented was to investigate some of the affecting factors such as

- to what extent are flux and protein transmission influenced by pH and ionic strength
- will a low transmembrane pressure help to improve protein transmission.

The ability of sodium hydroxide, hydrogene peroxide, sodium perborate, protamine sulfate and Terg-A-Zyme as potential membrane cleaners was to be examined.

Microporous membranes of different materials will be investigated if they are suitable to concentrate fermentation broths and to recover intracellular enzymes from yeast and cell debris.

A theoretical approach will be developed to describe the long-term loss of transmembrane permeability.

CHAPTER II

MATERIALS AND METHODS

1. MATERIALS

1.1 Filter Modules and Membranes

A number of the experiments were carried out with a hollow fibre membrane cartridge manufactured by A/G Technology Corporation, Massachusetts and distributed by Memtech (UK). The membrane was made of polysulphone and has been modified to be hydrophilic. The manufacturers would not provide any information on the type of treatment used. The first three letters in the membrane code characterise it as a cross-flow microfiltration type, the first figure indicates the nominal pore size in 100 nm, the next letter identifies the inner diameter of the hollow fibres and the last figure specifies the dimensions of the housing. CFP-2D-3 (Fiber inner diameter:0.75mm; housing outer diameter:3/8") and CFP-2E-3 (Fiber inner diameter:1mm; housing outer diameter:3/8") cartridges with a membrane area of 100 cm^2 and 75 cm^2 respectively and a pore size of 0.2 μm were employed.

A 150 mm diameter thin channel flow cell described elsewhere (Velicangil, 1976) was used for another set of experiments. The channel dimensions were modified to a width of 13.4 mm and a height of 2.4 mm. The effective membrane area of the system was 14.28×10^3 mm^2. Design details of the thin channel flow cell are given in Appendix III.

Different membrane types, shown below, were supplied by Domnick Hunter Filters. The membranes were in the form of sheets and were cut to the required size.

Aspor^{TM} membranes are asymmetric and consist of mixed esters of cellulose. Nypor^{TM} membranes are made of nylon and hydrophilic in nature. Cellulose acetate membranes which are naturally hydrophilic, are described to be best protein compatible. A polytetrafluoroethylene membrane was supplied by Gore-Tex. The highly hydrophobic material was wetted before use with absolute ethanol to allow permeation at low pressures.

The membranes had pore sizes of either 0.2, 0.45 or 0.6 microns.

1.2 Biologicals and Chemicals

Three forms of whey protein were donated by Bio-Isolates Ltd at Johnstown Creamery, Carmarthan. An ultrafiltrated eluate was supplied at 15% protein by weight, concentrated eluate at 28% and spray dried BiPro(TM) at 95% protein powder by weight. The analytical report, supplied by the company gave a protein composition of 16% α-lactalbumin, 80% lactoglobulin and 4% BSA for the eluate and the protein powder.

Both protein solutions were kept frozen and defrosted as required at room temperature. The whey powder was stored in a dry place. All the material was discarded after each run. Escherichia coli B/r cultures were given by the School of Pharmacy, University of Bath. Bakers yeast was provided by the School of Biochemistry, University of Bath.

Other biologicals and chemicals were purchased from commercial companies and are listed in Appendix II. The water used was Bath City water, treated by reverse osmosis in a Millipore laboratory unit.

2. THE MICROFILTRATION UNIT AND MEASUREMENT SYSTEM

Figure 2 shows a schematic layout of the filtration and measurement system. A photograph to scale of the measurement system is given in Appendix III. The filtration unit and the measurement system will be described separately.

2.1 The Microfiltration Unit

The system was operated in a batch mode with retentate recirculated by a Stuart Turner stainless steel centrifugal pump. The reservoir vessel was held in a constant temperature bath which was equipped with a thermostatically controlled heater as well as a cool unit. The piping network consisted of translucent reinforced PVC tubing. The inlet and outlet pressure gauges in the system were modified prior to use. Their base plates, made of cast iron as connection material ,was replaced by perspex. Two stainless steel needle valves, one installed prior to the filter housing, the second after the filter outlet, served to regulate the inlet and outlet pressure and the recycle flow of the system. The recycle flow was monitored by a built-in stainless steel float type rotameter. Prior to entering the microfiltration unit the feed was passed through a prefilter with a pore size of 20-25 μm which was selected to retain all particles like dust, metal fragments or lumps but to transmit proteins of interest readily. The inlet and outlet pressures were mostly 28 and 14 kPa respectively and a flowrate range of 0.4-0.5 ℓ/min. unless chosen to vary

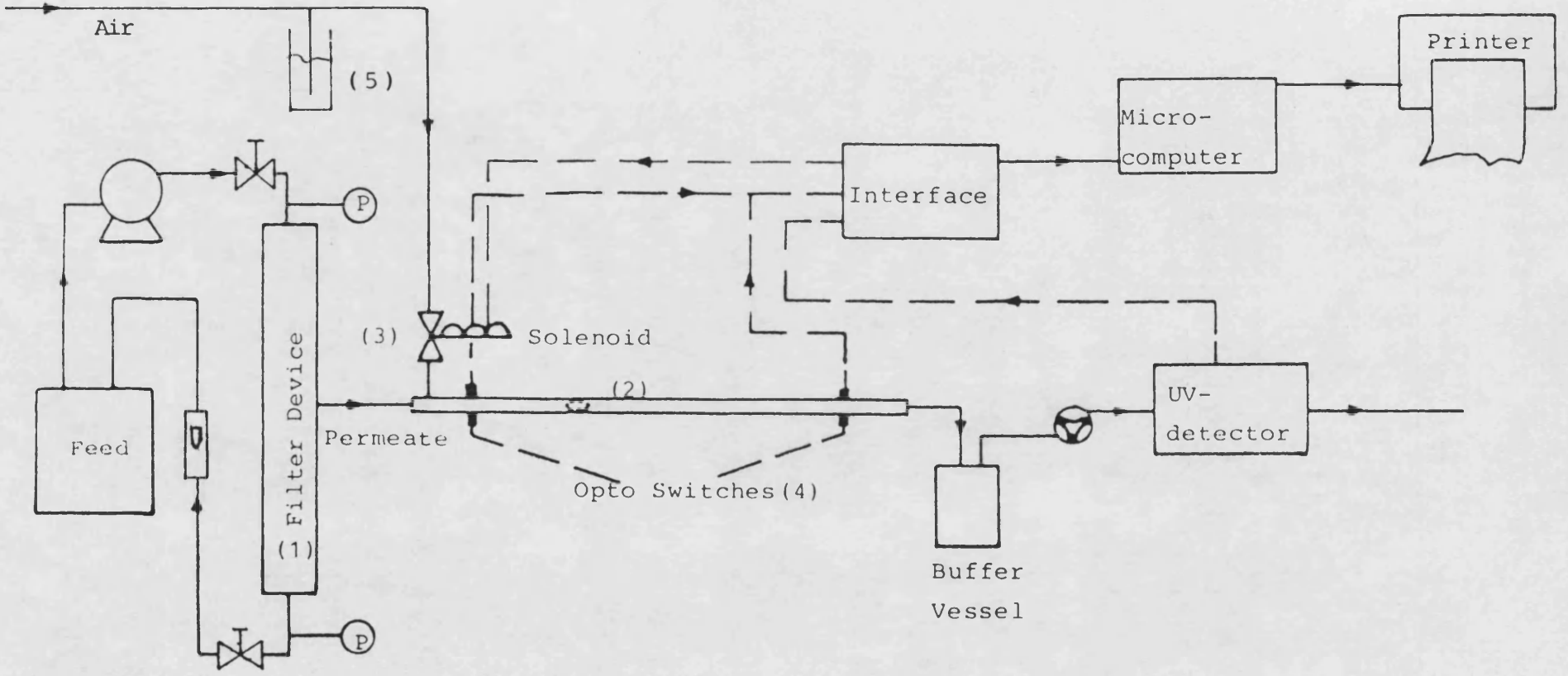


Fig.2: Experimental Set-Up with Flowmeter and UV-Detector Inserted to Allow Continuous Monitoring of Flux and Permeate Concentration

according to the purpose of the experiment.

All equipment and suppliers are listed in Appendix II.

2.2 The Microfiltration Measurement System

The filtrate outlet of the filter unit(1) was connected to a glass tube(2) of a known bore. An air inlet line followed by two TTL compatible opto-switches(4) each consisting of an LED and an integrated photodetector, were attached to the glass tube at a known distance apart.

Once the glass tube was filled with liquid, air from a low pressure reservoir was injected one bubble at a time. The leading edge of the bubble was detected by the two sensors as it passed along the tube. The software was modified so that the trailing edge of the bubble was ignored.

The size of the bubble was controlled by the time during which the airline was opened by a solenoid valve(3) which also controlled the time interval between two bubbles. All the sensors and controllers were connected to a BBC B microcomputer via an interface constructed in the laboratories. The computer was programmed to time the passage of the air bubble and to calculate the corresponding flux with the prior knowledge of the tube volume.

The permeate passed from the timing tube through a small buffer vessel from which it was picked up by a peristaltic pump and delivered to a UV cell. In cases where the protein concentration was beyond the UV-detection limits a multichannel peristaltic pump and a second buffer vessel with water were used to dilute the protein solution by a known factor.

The output of the UV unit was connected via an interface to the analogue input of the computer. From the data generated the computer software was developed to allow an on-line display of both flux and permeate protein concentration as a function of operating time. This could be dumped to the printer at will. Readings could be taken at intervals as short as 20 seconds although, apart from the initial stages of the flux decline, the intervals chosen were much longer.

A listing of the computer program to control the air injection, to time the passage of the air bubble, to record the output of the UV and to process the data can be found in Appendix I.

The accuracy of the flux measurement depends entirely on the bubble size. If it is too small the bubble will adhere to the glass wall, hence it will be much slower than the liquid flow. It is also important that the bubble size remains constant once the system has been calibrated. The size of the bubble is determined by the solenoid valve and the air pressure behind the solenoid, which has to be slightly higher than the pressure in the glass tube (atmospheric pressure) and more importantly, it has to be constant.

If the pressure increases during the time the airline is closed, the next injected bubble will vary in size or more than one bubble may be injected. This problem was solved by fitting a water filled column[5] between the pressure reservoir and the solenoid. The resulting pressure is determined by the water head (400 mm). A constant pressure system was achieved by allowing the excessive air to escape into the atmosphere.

The computer measured flux was calibrated against the stopwatch/volumetric vessel method to be accurate. The analogue input was calibrated against BSA standard solutions in a flow range of 0.05–15 ℓ/h to monitor protein concentrations in the range of 1–7.5 g/ℓ. Measuring such high protein concentrations necessitated the fitting of a UV detector with a 1 mm optical path flow cell.

All specifications and suppliers can be found in Appendix II.

3. BIOCHEMICAL ASSAYS

3.1 Optical Density

3.1.1 DNA at 260 nm

The amount of DNA in a clear solution was estimated by measuring the ultraviolet adsorption at 260 nm. The samples were read in a UV spectrophotometer against a blank which contained all components of the sample solution but the protein. Assaying DNA was necessary because its presence caused increased protein readings at 280 nm.

3.1.2 Protein at 280 nm

The amount of total protein in clear solution was estimated by measuring the ultraviolet adsorption at 280 nm. The samples were read in a UV spectrophotometer against a blank which contained all components of the sample solution but the protein. The optical density assay is very convenient and sufficiently sensitive for the present experiments using 1 cm path length cell. Protein concentration was calculated in mg/mℓ as follows (Racker,1955):

$$(1.55 \times (O.D._{280})) - (0.76 \times (O.D._{260}))$$

The relative protein transmission was then calculated:

$$\frac{\text{Concentration in permeate}}{\text{Concentraion in initial feed solution}}$$

3.1.3 Cell Density at 650 nm

The microbial cell density of the fermentation broth was estimated by measuring the adsorption in the visible light range. To 1 mℓ of the culture 3 mℓ 10% formalin was added. The optical density was read in a spectrophotometer and the cell density obtained from the relationship

$$E_{650}0.6 = 1 \text{ mg/m}\ell.$$

3.2 Enzyme Activity

3.2.1 β-Galactosidase Assay

The assay is described by Hamilton (1982). The cloudy retentate solution was centrifuged prior to assay whilst the clear permeate was used without treatment. 0.15 mℓ samples were withdrawn and diluted with distilled water to a total volume of 1 mℓ.

The sample tubes were placed in a water-bath at 30°C for 30 minutes. 0.2 mℓ 13.3 mmol o-nitrophenyl-β-D-galactoside (ONP) was added and incubated for exactly 10 minutes. The reaction was stopped with 0.5 mℓ 1M sodium carbonate. Each sample was read at 550 and 420 nm against a water blank.

E_{550} gives a measure of light scattering due to cells etc and E_{420} gives a measure of light scattering and the yellow colour of o-nitrophenol in alkaline solution. The measure of ONP is given by the relationship

$$E_{420} - (E_{550} \times 1.65)$$

The relative enzyme transmission was then calculated.

$$\frac{\text{Activity of permeate}}{\text{Activity of initial feed solution}}$$

3.2.2 Alcohol-Dehydrogenase Assay

Alcohol-dehydrogenase catalyses the oxidation of ethanol by NAD (Nicotine-adenine-dinucleotide) to give the corresponding aldehyde and $NADH_2$.

The method of assay depends on the difference in the extinction coefficients of NAD and $NADH_2$ at 340-366 nm due to the chromophoric structure of $NADH_2$ which is absent in NAD. The standard conditions are based on those described by Racker (1955). The solutions required are:

Sodiumpyrophosphate buffer 0.06M; pH 8.5
NAD 0.015M
Ethanol 3M

0.3 mℓ samples were withdrawn and diluted to a total volume of 5 mℓ. 0.1 mℓ of the solution was placed in a cuvette together with 0.5 mℓ buffer, 0.1 mℓ NAD and 2.2 mℓ distilled water. The spectrophotometer set at 340 nm was adjusted to zero using the above mixture. 0.1 mℓ ethanol was added and the first reading taken within 15 seconds. Further readings were taken at 15 second intervals for the first two minutes and then every 30 seconds for a total reaction time of four minutes.

The optical density versus time was plotted and the initial velocity calculated from the slope of the linear curve. Enzyme activity was calculated in international units (IU) and expressed as specific activity which is defined as IU per mg protein. The relative enzyme transmission was then calculated:

$$\frac{\text{Specific activity of permeate}}{\text{Specific activity of initial feed solution}}$$

4. FERMENTATION AND CELL DISRUPTION

4.1 β-Galactosidase production

4.1.1 Fermentation

Escherichia coli B/r was grown in a defined medium. The procedure was based upon the method of Hamilton (1982) and adopted to enhance the production of the intracellular enzyme β-galactosidase.

Solution A:
15.1 g KH_2PO_4
2.2 g $(NH_4)_2SO_4$
1 mℓ of 0.006 g/mℓ $FeSO_4 \times 7\ H_2O$
1ℓ H_2O
adjusted with NaOH to pH7

Solution B:
0.2 g $MgSO_4 \times 7H_2O$
100 mℓ H_2O

Solution A and B were both autoclaved separately for 15 minutes at 1 bar. 20% lactose and 0.05% thiamine solutions were filter sterilised using a 0.2 μm autoclavable Whatman filter.

90 mℓ of Solution A, 10 mℓ of Solution B, 1 mℓ lactose and 0.1 mℓ thiamine were mixed under aseptic conditions and the micro-organism inoculated. The start culture was allowed to grow overnight in a shaking incubator set to 37°C. The actual fermentation was carried out with 9 ℓ of Solution A, 900 mℓ of Solution B, 90 mℓ lactose, 1 mℓ thiamine and inoculated with 100 mℓ of the start culture. The mixture was fully aerated and agitated at 200 RPM and left to grow at 37°C until the optical density gave a reading at E_{650} of 0.48 which was equal to a density of 0.8 mg/mℓ. The fermentation broth was then stored in a cold room at 8°C until processed, usually not longer than one night.

4.1.2 Cell Disruption

The E coli cells were disrupted by ultrasonic energy. To prevent enzyme denaturation due to local overheating, portions of only 40 mℓ were used at a time and kept in an ice bath during the treatment. The suspension was exposed in the ultrasonic bath for 15 seconds and then allowed to cool down for the same length of time. The total time of procedure was two minutes. The suspension was then brought to a total volume of 1.5 ℓ potassium phosphate buffer solution.

4.2 Alcohol-Dehydrogenase production

4.2.1 Disruption of Bakers Yeast

Bakers yeast was air-dried at 37 °C for 48 hours.

4.2.2 Resuspension of Yeast Fragments

The procedure is described by Racker (1955). 25 g of air-dried bakers yeast were suspended in 25 mℓ of 0.066 M Na_2HPO_4 and 0.001 M EDTA. The mixture was stirred with a wooden dowel to work out the lumps. A further 50 ml buffer solution was added slowly and under constant stirring so that the final mixture was smooth. The flask was kept in a shaking bath at 37 °C for two hours and then at room temperature overnight. The suspension was brought to a total volume of 1.5 ℓ phosphate buffer solution prior to filtration.

5. EXPERIMENTAL DESIGNS

5.1 Continuous Microfiltration Experiments

In all continuous experiments 1.5 ℓ feed solution was kept in a constant temperature bath at 10°C to dissipate frictional heat due to the pump and avoid bacterial degradation of the proteins (Tanny et al, 1982). The feed pipe-line was filled with the solution to expel most of the trapped air prior to the starting of the pump. The filter housing was filled with water to prevent an immediate direct contact of the protein solution with the membrane. Experiments performed in A/G Technology's own laboratories showed a more dramatic initial flux decay when the run was started up on an unfilled housing (Houldsworth, private communication). With the starting of the pump the internal clock of the PC was reset to zero and the first measure point taken as soon as the operating pressure was adjusted.

All experiments carried out with the hollow fibre cartridge operated at Re = 6500 - 8500 or equivalent to a recycle flow rate of 0.4 - 0.5 ℓ/min.

All experiments performed with the thin channel flow cell system operated at Re = 1200 - 2800 or equivalent to a recycle flow rate of 0.7 - 1.7 ℓ/min.

5.2 SEPARATION OF PROTEINS AT DIFFERENT pH AND IONIC STRENGTHS

The whey eluate and the concentration were diluted approximately to the required concentration and analytically determined.

Protein powder like BSA, casein, ovalbumin and BiPro^{TM} was weighed, dissolved and the concentration analytically determined. The coarse undissolved lumps of the BiPro^{TM} solutions were removed prior to use.

Usually the ionic strength and pH was adjusted before the run. Some experiments were chosen to demonstrate the immediate response of the flux and protein transmission to pH changes. pH was adjusted by adding small amounts of HCℓ or NaOH without stopping the pump and samples were then taken from the feed reservoir to measure the pH. This procedure was necessary to guarantee that variations in flux and transmission arose due to the altered environment and not due to changed hydrodynamic conditions. All cheese whey and protein except BiPro^{TM} experiments were carried out with the hollow fibre cartridge.

5.3 LOW PRESSURE EXPERIMENTS

One set of runs was started at a pressure of 600 Pa and the initially measured flux maintained by increasing the transmembrane pressure in small intervals.Another series of experiments was performed at a transmembrane pressure 1130 Pa.Since the Budenberg gauges were not precise enough to measure such a low pressure a waterhead installed after the permeate outlet served as a pressure indicator.

5.4 CELL HARVESTING AND β-GALACTOSIDASE SEPARATION

Cell harvesting was performed with the 150 mm diameter thin-channel flow cell. Asypor^{TM} and Nypor^{TM} membranes with a pore size of 0.2, 0.45 and 0.6 microns were applied. The operating pressures were 14 kPa and 98 kPa. A 10 ℓ fermentation broth was concentrated to a final volume of 800 mℓ.

The separation of β-galactosidase was performed in the same way but mostly at 14 kPa unless stated differently.

5.5 YEAST DEBRIS REMOVAL

The 150 mm diameter thin-channel flow cell was applied and mounted with PTFE or cellulose acetate membranes. A pore size of 0.45 μm was chosen. The operating pressure was 14 kPa.

5.6 CLEANING

Routine cleaning was done by recycling a Terg-A-Zyme detergent solution 3 g/ℓ in weight at 55°C for two hours at a pressure of 20 kPa.

The standard flux was measured at 20 kPa transmembrane pressure, at 10°C and a flow rate of 0.4 ℓ/min with a 0.1 M sodium chloride solution.

Terg-A-Zyme was preferred to NaOH because it was believed that the enzyme-active detergent harms the membrane less.

The conditions of different cleaning experiments are listed in Table 6.

TABLE 6: Cleaning Chemicals and Conditions

Cleaning Chemical	Concentration g/ℓ	Temperature °C	Time (hrs)
H_2O_2	1	55	5
NaOH	1	55	2
$NaBO_3*4H_2O$	1	55	2
Protamine sulfate	2	10	2

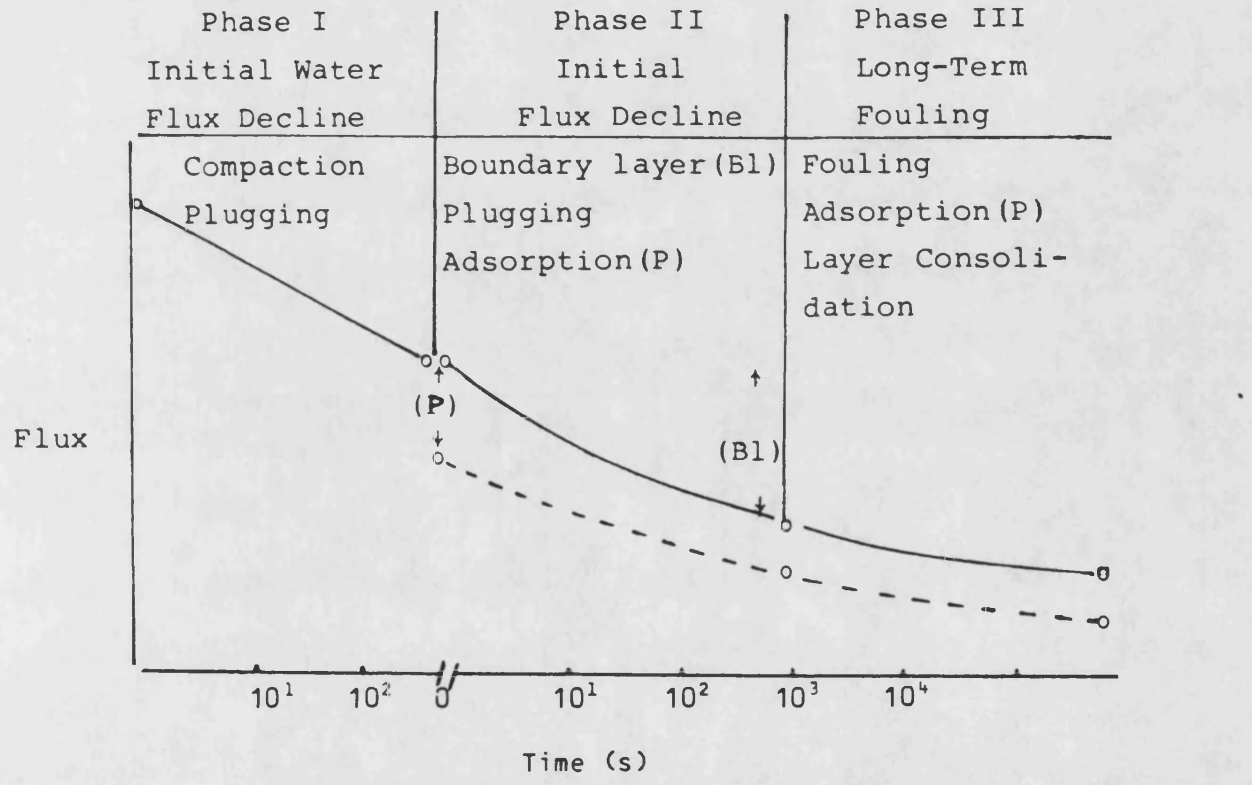
The applied transmembrane pressure was in all cases 20 kPa.

CHAPTER III

MATHEMATICAL MODEL

The flux decline of an ultra- and microfiltration membrane can be divided into three phases as depicted in Fig.3.

Fig.3 A Typical Flux History of Membranes



Phase I is the period of membrane usage prior to filtration, during which the membrane may be washed and have its water flux measured. An initial drop in flux is generally observed. Possible reasons are contamination of the used water, hydrolysis and compaction of the membrane or electrostatic effects, which will be explained in more detail later.

Phase II is principally caused by the build-up of a polarised layer which has been calculated to be complete within a few seconds (Howell and Veligancil, 1982). Simultaneously, pores become plugged and solute such as proteinaceous material adsorbs to

the membrane. The concentration polarisation of the membrane surface accelerates plugging and adsorption.

There is further a flux decline which is irreversible and can be observed over hours and days. This long-term decline has been termed fouling and determines phase III entirely. It is believed that the phase III flux decline is a consequence of a deepening of the fouling layer at a rate dependent on the solute concentration at the membrane surface. Velicangil (1979) first described the long-term fouling by relating the solute concentration at the wall to the increasing layer thickness:

$$\frac{d\ell}{dt} = K_R C_s^n$$

The same year Nakao et al (1979) also established a relationship between the limiting wall concentration and the resistance as a consequence of the formed layer.

Since the layer thickness is proportional to the resistance the empirical equation can be modified as follows:

$$\frac{dR_A}{dt} = k_R C_s^n$$

The objective of this chapter is to investigate the applicability of a long-term fouling model to different proteinaceous systems at various operating conditions and to determine the reaction rate constant n at which the proteins build up into a fouling layer and the order of reaction k at which the fouling process takes place.

1. CALCULATION OF THE LONG-TERM FLUX DECAY

Flow through cylindrical pores can be described by Darcy's law:

$$Q_F = K_F \frac{A_m \Delta P}{\mu \Delta X} \qquad (1)$$

where Q_F = flow through pores

K_F = filtration constant, also called Darcy's coefficient

A_m = total membrane area

ΔP = transmembrane pressure drop

ΔX = apparent thickness of the membrane

This has been applied to packed beds also where flow channels are neither cylindrical nor straight.

If the permeability K is inverted to a resistance (K=1/R) and the resistance apportioned between the adsorbed fouling layer (R_A) and the membrane (R_m) Darcy's law becomes

$$J = \frac{\Delta P}{\mu(R_m + R_A)} \qquad (2)$$

R_A could be itself related to the layer thickness ℓ and its permeability K_A

$$R_A = \frac{\ell}{K_A} \qquad (3)$$

Flux can also be described by the concentration-polarisation model:

$$J = k_m \ell n \frac{C_s}{C_b} \qquad (4)$$

when C_s is the concentration of solute at the membrane surface. If flux becomes limiting then

$$C_s \leq C^*$$

Since long-term fouling is generally observed over hours and days, although it commences at time zero together with the concentration polarisation, an incorporation of the time dependency is required:

$$J(t) = \frac{\Delta P}{\mu(R_m + R_A(t))}$$

or
$$R_A(t) = \frac{\Delta P}{\mu J(t)} - R_m \qquad (5)$$

Differentiating eqn (5) yields

$$\frac{dR_A}{dt} = -\frac{\Delta P J^{-2}}{\mu} \frac{dJ}{dt}$$

or

$$\frac{dJ}{dt} = -\frac{J^2 \mu}{\Delta P} \frac{dR_A}{dt} \qquad (6)$$

The empirical relationship

$$\frac{d\ell}{dt} = K_R C_s^n \qquad (7)$$

describes the rate of growth of the adsorbed layer and since

$$\frac{d\ell}{dt} \propto \frac{dR_A}{dt}$$

can be modified to express the growth of resistance:

$$\frac{dR_A}{dt} = k_R C_s^n \qquad (8)$$

combining eqns (6) and (8) and substituting C_s by (4) leads to the final equation describing the long-term flux decline.

$$\frac{dJ}{dt} = - \frac{J^2 \mu k_R C_b^n \exp[nJ/k_m]}{\Delta P} \qquad (9)$$

The linkage of the two models of concentration polarisation and resistance in series offers the opportunity to analyse the time dependent flux decline taking into account the transmembrane pressure, the resistance due to the fouling layer, the hydrodynamic conditions and the bulk concentration.

The increase of the resistance over a longer period is dependent on the concentration of the bulk and the two parameters k_R and n which will be determined, inter alia, by the rheology of the solution: ie the type of the solute, pH, ionic strength and temperature.

Velicangil (1979) found n=2 for Cheddar cheese whey and Bovine serum albumin. Nakao (1979) obtained n=1.7 for Polyvinyl alcohol and ovalbumin but different values of k_R. PVA 224 yielded $k_R = 2 \times 10^3$ and ovalbumin $k_R = 4.5 \times 10^2$.

Values of k_R and n were determined for Bovine serum albumin at different pHs from data in the literature (Aimar et al, 1986), and are listed in the following table:

pH	k_R	n
2	1.2×10^7	1.5
4.7	1.8×10^8	1.3
7.2	2×10^7	1.5

It is obvious that n and k_R depend on the nature of the proteinaceous solution and a consequent step was to determine k_R and n at various operating and rheological conditions.

A computer program was used to process data using non-linear least squares optimisation to evaluate the values of k_R and n for a set of data. The initial values of parameters were fed into the first order differential equation (9) which was solved with a numerical integration method. The least square criterion was applied to find values of k_R and n

giving the best fit to the experimental results. The listing of the program can be found in Appendix I.

The mass transfer coefficient of the thin channel flow cell for solute transport away from the membrane surface was calculated from the Dittus-Boelter correlation for turbulent flow in thin channels, given in dimensional form as:

$$k_m = \frac{0.02 \quad Q^{0.8} \; D^{0.67}}{b \; w^{0.8} \quad \nu^{0.47}}$$

where Q is the volumetric flow rate (2.67×10^{-5} m^3/s); b is the channel depth (2.4×10^{-3} m); w is the channel width (13×10^{-3} m); ν is the kinematic viscosity (1.3×10^{-6} m^2/s) and the diffusivity value used was 10.4×10^{-11} m^2/s. For the above system $k_m = 7.1 \times 10^{-6}$ m/s with Re = 2600.*

The same correlation was applied to the hollow fibre system with

$$k_m = \frac{0.02(4Q)^{0.8} \; (D)^{0.67}}{(\pi d^2)^{0.8} \; d^{0.2} \quad \nu^{0.47}}$$

where $Q = 6.5 \times 10^{-6}$ m^3/s and the channel diameter $d = 0.75$ m^{-3},
$k_m = 8.7 \times 10^{-5}$ m/s with Re = 8500*.

It can be argued that turbulent flow correlations should not be applied for Re < 10000 but it is generally accepted that the transition from laminar to fully developed turbulent flow is complete at Re = 2000 (Porter, 1972), especially in small systems where entrance effects are significant.

The dynamic viscosity $\mu = 1.3 \times 10^{-3}$ kg/m s was assumed to be similar to that of water and the transmembrane pressures ΔP were 1.1×10^5 Pa for the thin channel flow cell and 1.1×10^4 Pa for the hollow fibre module.

The bulk concentration C_b varied according to the experiment.

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* In cases where k_m differs it will be expressly mentioned

2. DETERMINATION OF THE PARAMETERS k_R AND n

Equation (9) describing the long-term fouling is not applicable to the initial phase of rapid flux decline which is mainly determined by concentration polarisation followed by pore plugging and fast initial protein adsorption to the membrane surface. It rather reflects a further solute deposition at a much slower rate than the previous adsorption stage. Superimposed but not explicit in the model will be effects of the fouling step exhibiting an increasing specific resistance with time which results in a slow flux decline over hours or even days. Equation (9) was therefore integrated from the time when the initial phase was assumed to be complete and the results compared with the experimental results.

2.1 Single Protein Systems

Figures 4 and 5 illustrate the long-term flux decay during microfiltration of bovine serum albumin (BSA 1.5 g/ℓ) at different pH and ovalbumin (4g/ℓ) in different buffer systems.

The values of n and k_R are listed in Table 7.

TABLE 7 n and k_R of BSA and Ovalbumin

Solute	pH	Buffer 0.1 Mol/ℓ	k_m m/s	k_R	n	ϵ	$\frac{\epsilon}{m-3}$	
BSA	2.8	-	6.6×10^{-5}	2.4×10^{6}	4.5	8	0.8	Fig.4
	4.7	-	6.6×10^{-5}	2.4×10^{8}	1	10	0.4	
	9.2	-	6.6×10^{-5}	2.2×10^{7}	0.2	5.9	1.2	
Ovalbumin	9.2	-	2×10^{-4}	6.7×10^{6}	1.9	59	9.9	Fig.5
	9.2	Potassium-Phosphate	2×10^{-4}	1.7×10^{7}	1.7	5.3	1.1	
	9.2	NaCℓ	2×10^{-4}	2.8×10^{6}	3.2	3.5	0.5	

The best fit to the experimental data was found for BSA filtration at pH=4.7 which is the I.E.P. of this particular protein and for the buffered ovalbumin systems. A possible interpretation can be given in terms of electrical interactions. In unbuffered systems and those different from the I.E.P. the proteins possess a net positive or negative charge causing either repulsion or attraction between the adsorbed and free proteins and also between the proteins and those areas of the membrane which were not covered during the initial rapid adsorption phase. With time more and more proteins of the fouling layer will have the same net charge as those being still in solution, repulsion becomes more likely and a further growth of the layer hence an increase in resistance will be retarded. Coupled with this the concentration at the membrane decreases with decreasing flux due to enhanced back diffusion of solute into the bulk solution. The predicted flux is therefore lower than the observed one.

In buffered systems and those with a pH close to the I.E.P. electrical effects are much weaker or even absent and the model predicts a flux decay fitting the observed data more precisely.

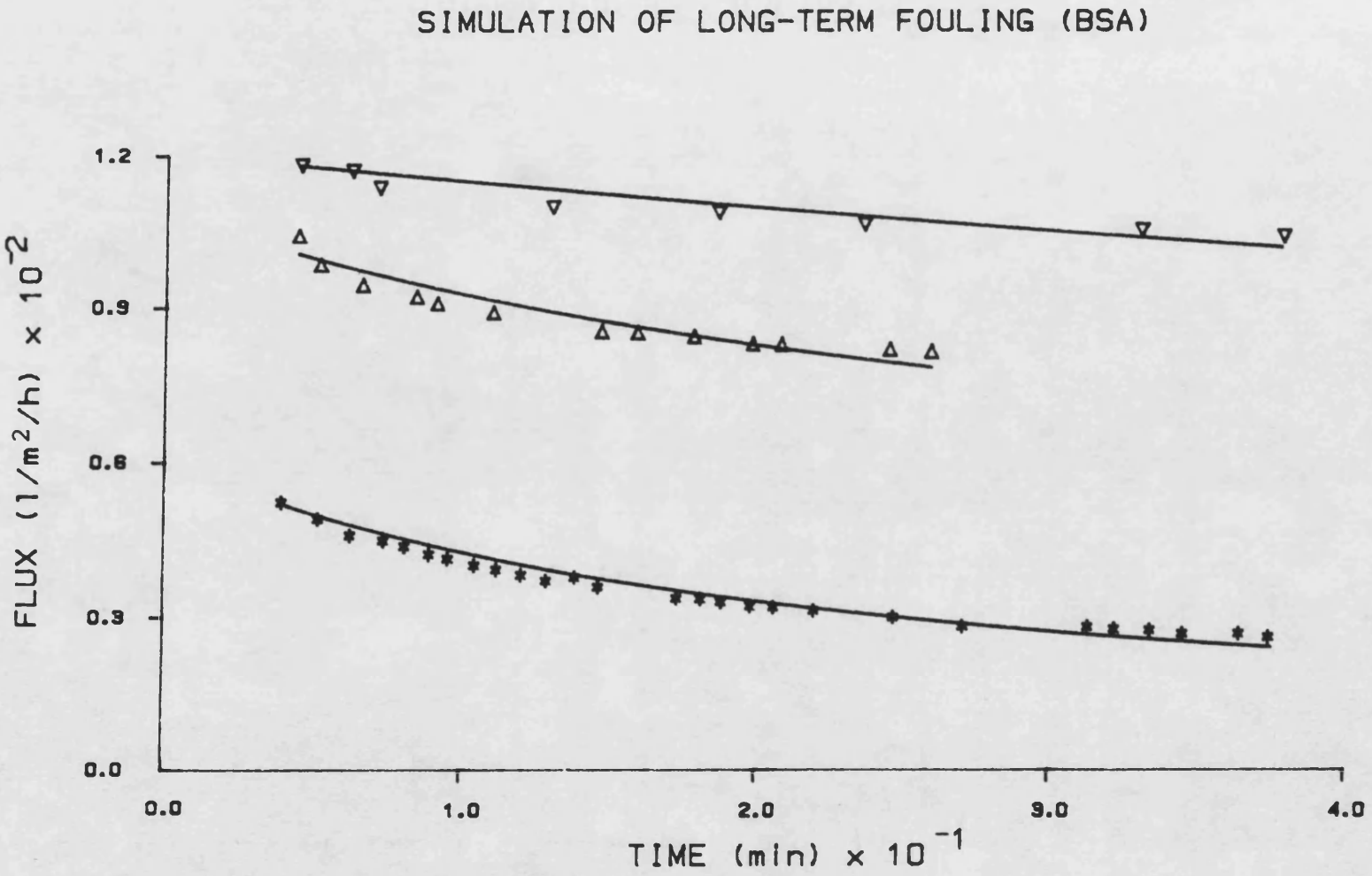


Fig.4: CFP-2E-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20kPa; T=10°C; Feed: BSA; C_b=1.5 g/ℓ; Δ pH=2.8; * pH=4.7; ∇ pH=9.2;

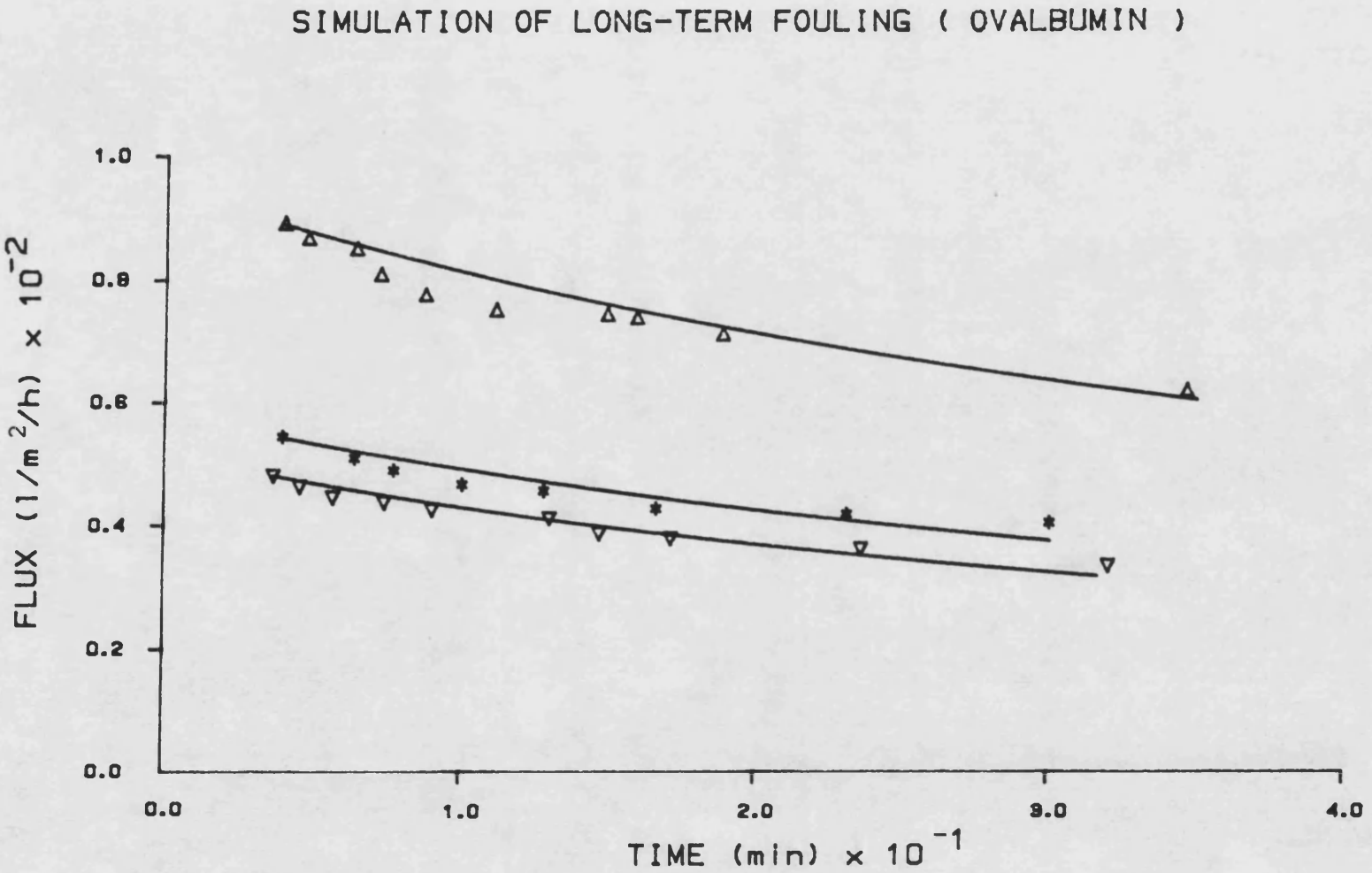


Fig.5: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20kPa; T=10°C;
Feed: Ovalbumin: C_b=4 g/ℓ; pH=9.2:
Δ no salt; * 0.1 M Potassium Phosphate; ▽ 0.1 M NaCℓ ;

2.2 Mixed Protein Systems

The different values of n and k_R which are the result of cheese whey microfiltration at two different bulk concentrations, varying pH and ionic strength, are listed in Table 8.

TABLE 8 n and k_R of Cheese Whey

C_b g/ℓ	pH	NaCℓ	k_R	n	ϵ	$\frac{\epsilon}{m-3}$	
13.4	3.5	-	1.7×10^8	1	1.8	0.3	Fig.6
13.4	4.2	-	2×10^7	1.8	2.7	0.4	Fig.6
15.1	5.2	-	8.5×10^7	0.4	9.0	1.0	
13.4	6.0	-	4×10^7	1.3	3.0	0.4	
13.8	8.2	-	1.6×10^8	0.3	8.8	1.8	
12.6	3.5	0.1M	1.7×10^8	0.4	8.4	1.1	Fig.7
16.6	4.2	0.1M	1.1×10^8	1.0	7.1	1.4	
13.2	5.2	0.1M	1.4×10^8	0.5	4.4	0.4	
13.0	8.5	0.1M	1.4×10^8	0.4	1.6	0.2	Fig.7
1.5	3.5	-	2.0×10^6	5.2	18.3	2.0	Fig.8
1.5	4.2	-	2.7×10^8	1.2	16.9	1.0	
1.5	5.2	-	3.5×10^7	2.1	12.5	0.5	Fig.8
1.5	8.5	-	3.3×10^6	7.4	17.6	1.6	Fig.8
1.5	3.5	0.1M	4.5×10^7	4.9	9.2	0.7	Fig.9
1.5	5.2	0.1M	6.5×10^7	5.2	9.1	0.6	Fig.9
1.5	8.5	0.1M	3.4×10^7	4.9	9.4	0.7	

Figs. 6, 7, 8, and 9 illustrate representative examples.

As with single protein solutions, the best fit is obtained for mixed protein systems with environmental conditions at which electrical effects are not dominating ie, at a pH around the isoelectric point and at a higher ionic concentration.

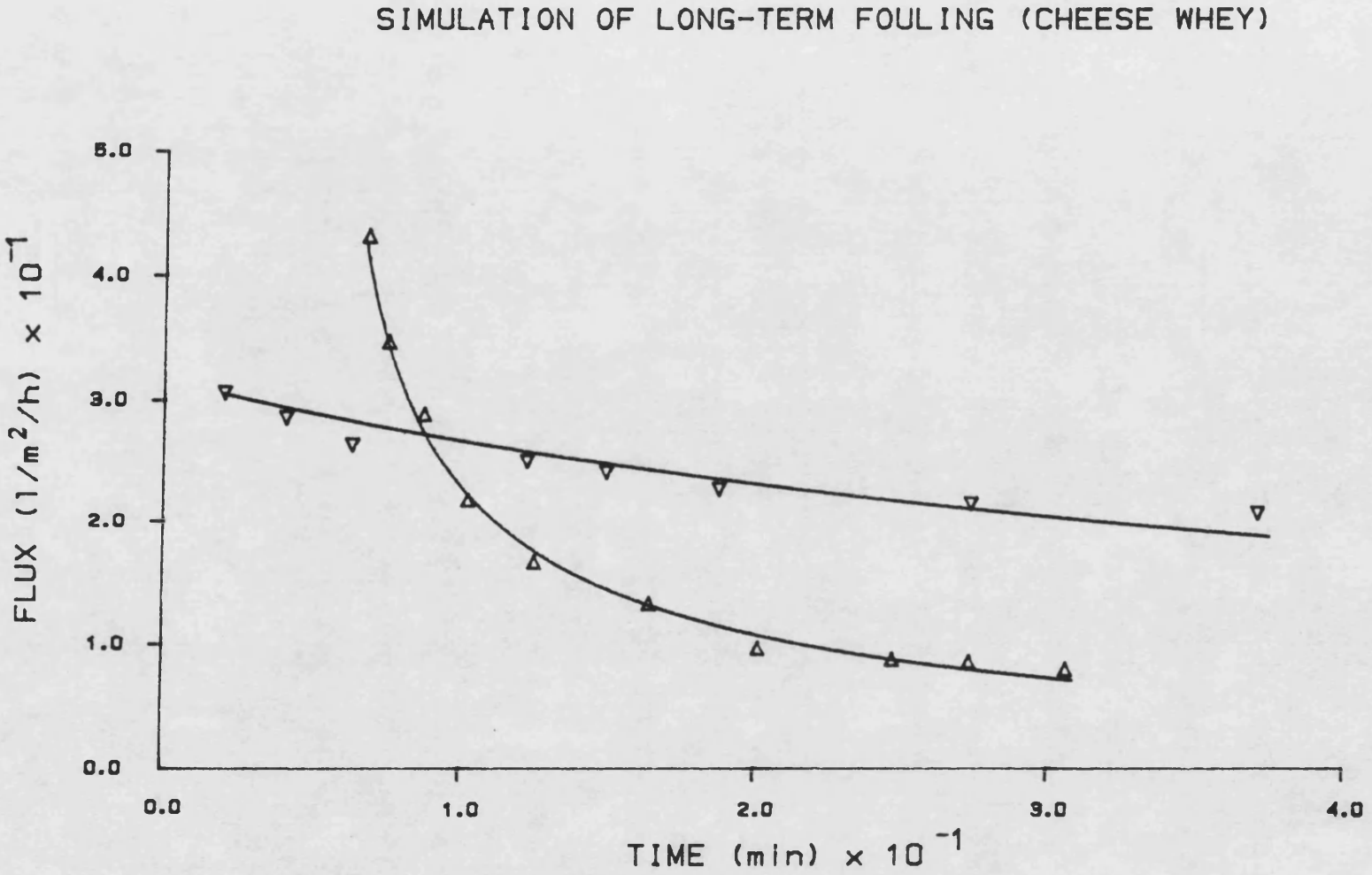


Fig.6: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20kPa; T=10°C;
Feed: Whey Protein; C_b=13.4 g/ℓ; ∇ pH=3.5; Δ pH=4.2;

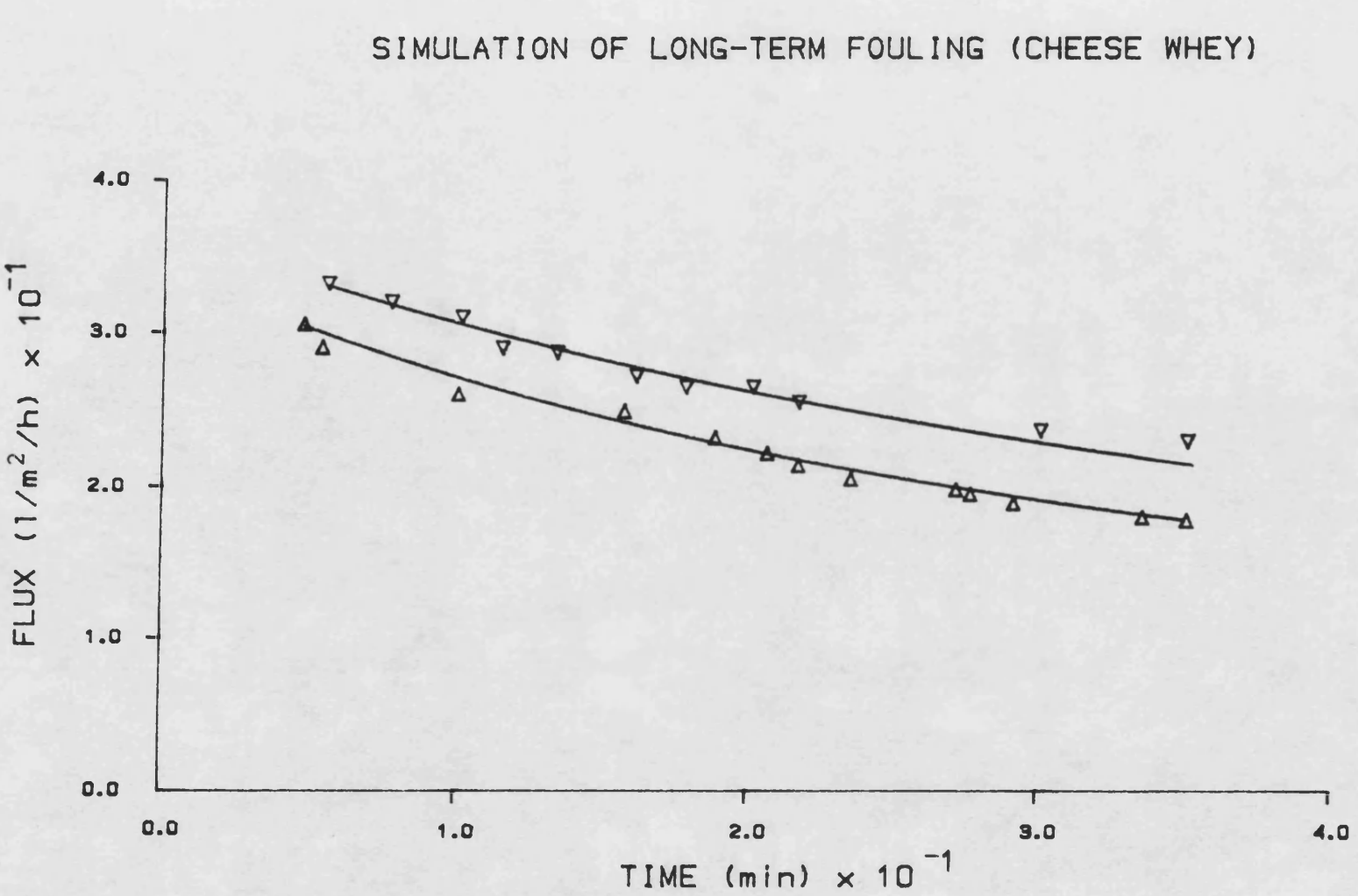


Fig.7: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20kPa; T=10°C; Feed: Whey Protein; 0.1 M NaCℓ; C_b=13.0 g/ℓ; Δ pH=3.5; ∇ pH=8.5;

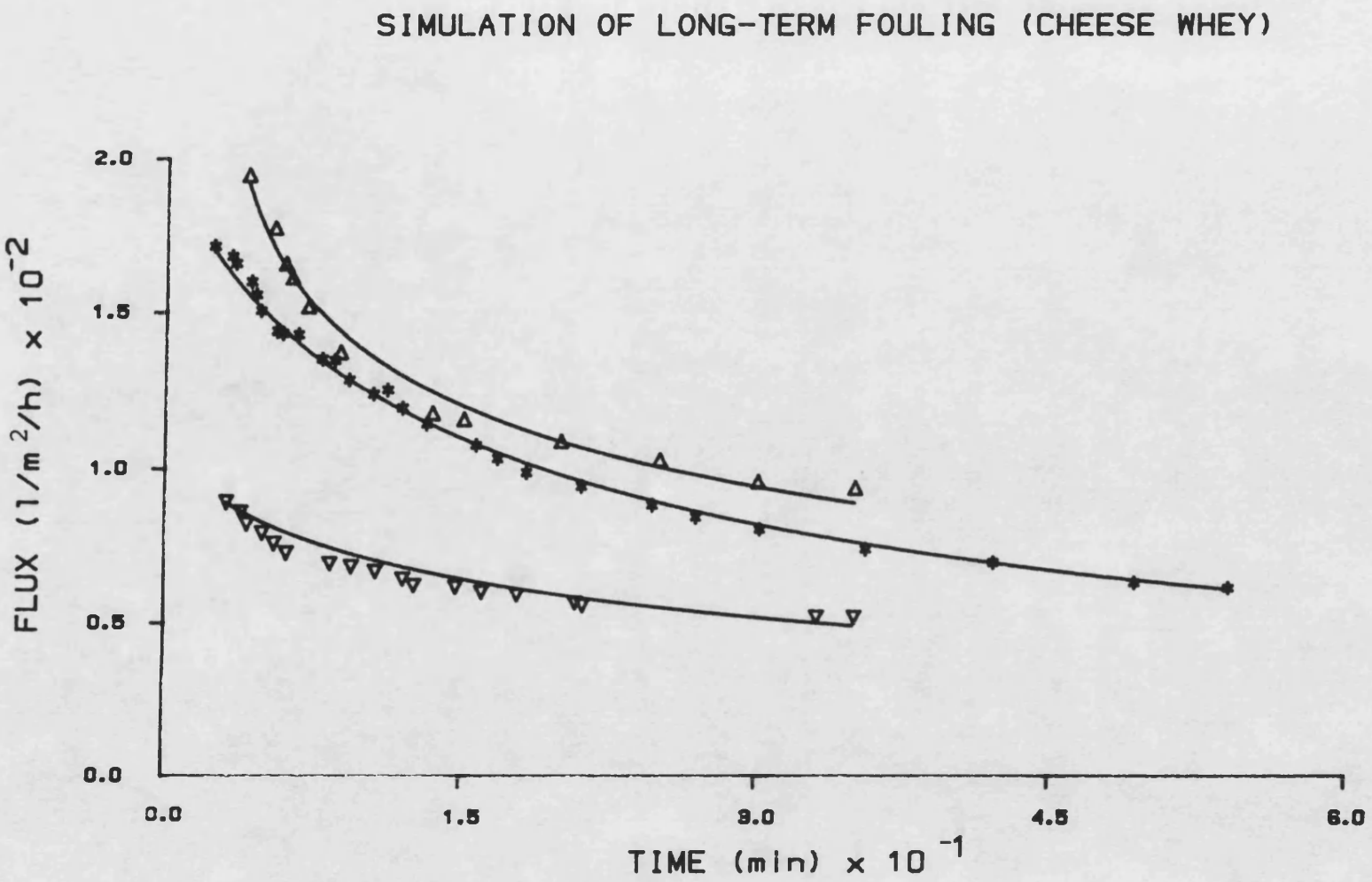


Fig.8: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20kPa; T=10°C; Feed: Whey Protein; C_b=1.5 g/ℓ; Δ pH=3.5; * pH=5.2; ∇ pH=8.5:

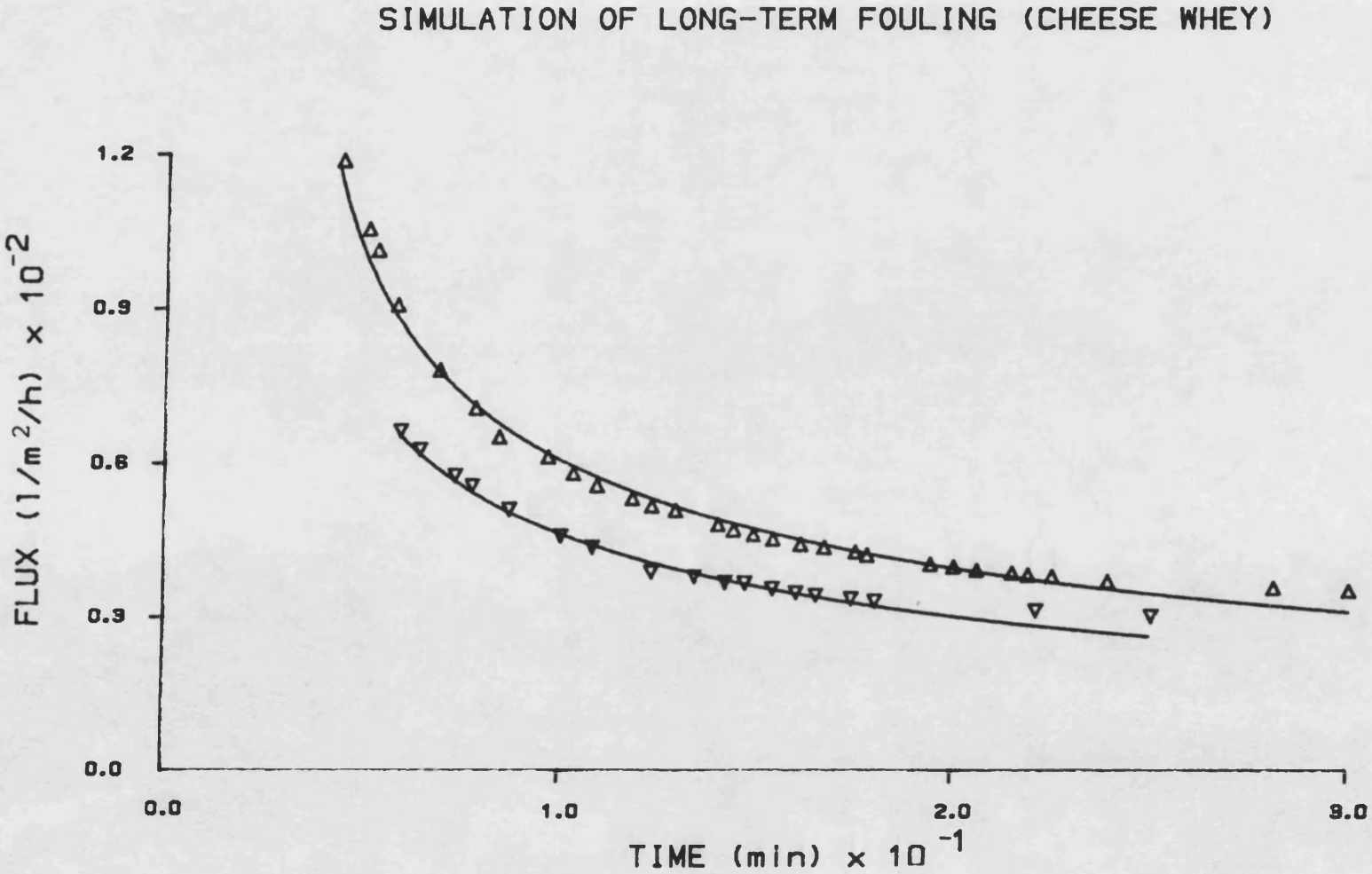


Fig.9: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20kPa; T=10°C; Feed: Whey Protein; C_b=1.5 g/ℓ; 0.1 M NaCℓ; Δ pH=3.5; ∇ pH=5.2;

Notable here is that the model holds for a wider range of pH. Best fits were yielded for pH=4.2 (Fig.6) and pH=5.2 (Fig.8). Cheese whey consists of α-lactalbumin, β-lactoglobulin and BSA each with its own I.E.P. causing a spectrum of pH during which lowest interactions due to electrical effects appear.

The flux prediction is also applicable to different bulk concentrations. Both systems investigated with C_b=13.4 g/ℓ (Figs.6,7) and C_b=1.5 g/ℓ (Figs.8,9) gave equally good fits.

2.3 Cell Suspensions

Simulations of long-term flux decline of fermentation broths like Escherichia Coli cell suspensions gave in general a good agreement with the experimental observations.

The calculated values of n and k_R for different membrane materials, pore sizes and cell concentrations are listed in Table 9.

TABLE 9 n and k_R of Escherichia Coli

Membrane	Pore size μm	E.coli conc. g/ℓ	k_R	n	ϵ	$\frac{\epsilon}{m-3}$	
Nypor™	0.2	0.83	3.7×10^7	1	9	0.4	Fig.10
	0.2	1.40	6.1×10^7	1.7	3.5	0.3	
	0.45	1.25	5.9×10^7	0.7	27.4	1.3	Fig.10
	0.45	2.30	3.8×10^7	1	37.9	1.9	
	0.6	1.0	3.6×10^7	2.1	3.5	0.2	Fig.10
Aspor™	0.2	0.83	5.3×10^7	1.4	3.6	0.2	
	0.45	1.2	2.9×10^7	1.3	4.6	0.2	
	0.45	1.9	3.8×10^7	1	2.8	0.1	
	0.6 *	0.8	5×10^7	0.4	6.0	0.4	

* km = 3×10^{-6}

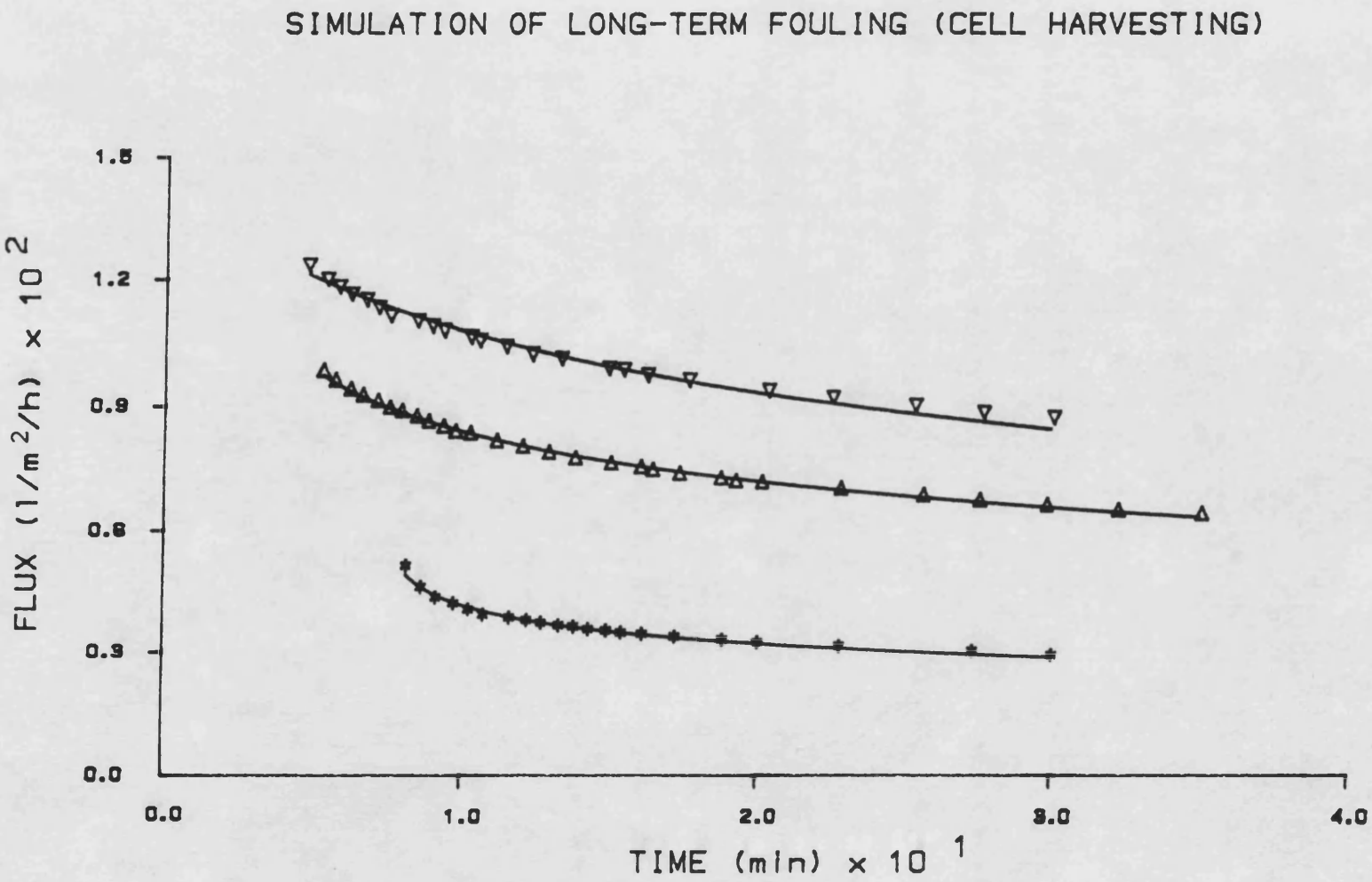


Fig.10: Nypor™ Membrane; Recycle Flow Rate=1.5 ℓ/min; ΔP=110kPa; T=10°C;
Feed: E.Coli; pH=7;
Δ C_b=0.83 g/ℓ; Pore size=0.2μm; ▽ C_b=1.25 g/ℓ; Pore size=0.45μm;
* C_b=1.0 g/ℓ, Pore size=0.6 μm;

Fig.10 shows the long-term flux predictions of cell harvesting performed with Nypor^{TM} membranes of different pore sizes. Compared to the single and mixed protein systems the model seems to describe the long-term fouling process during cell harvesting best. Unlike proteins, intact cells do not possess net charges and are therefore not subject to interactions between each other or with the membrane surface due to electrical effects. The long-term fouling is apparently determined by solute deposition on the membrane surface and a subsequent layer consolidation which are the main assumptions of the long-term flux model.

2.4 Cell Debris Suspensions

Attempts to apply the model to flux decrease during cell debris removal failed. A suspension of lysed cells contains various proteins, enzymes, DNA and differently sized cell wall fragments (because of ultrasonic destruction). The particles may be charged or uncharged and cover a range of size. In addition to elelctrical effects a continuous pore plugging, also inside the pores, is likely to occurr. This results in an extremely steep flux decay during the initial adsorption and pore plugging phase followed by a short period during which the flux levels off and remains almost constant thereafter. It therefore seems that the flux decline is mainly determined by pore plugging rather than a progressive solute deposition and enhanced resistance on which the long-term fouling model is based.

2.5 Discussion

The parameters k_R and n represent the reaction rate constant at which the proteins build up into a fouling layer and the order of reaction at which the fouling process takes place. To give a final conclusion with respect to n is somewhat difficult because the values seem to differ considerably. Neglecting cases of extreme pH conditions where proteins are strongly charged and also likely to be denatured and cases where membranes with a large pore size were applied and pore plugging is the major determining factor a general trend is recognisable. Most values of n are between 1 and 2 indicating a second order reaction. The assumption of a second order reation appears to be reasonable since the observations described in Chapter IV and those reported in the literature strongly suggest an interaction between the proteins in solution and the membrane itself which, after the initial fouling phase by absorption will have a protein surface.

CHAPTER IV

EXPERIMENTAL RESULTS AND DISCUSSIONS

This chapter on experimental results and discussions is divided into five parts. Part I is on the properties of the membrane itself. Part II is devoted to membrane fouling by protein under different environmental aspects. Part III highlights the beneficial effect of a low transmembrane pressure on protein transmission. Part IV is a contribution to the more commercial application of cross-flow microfiltration cell harvesting and recovery of intracellular enzymes. Part V discusses the relative efficacy of several cleaners applied.

PART I

The Properties of Membranes

The behaviour of CFP-2 membranes under different environmental conditions was investigated. The manufacturer recommended a maximum working temperature of 80 °C at pH7, a pH range of 2-11 at 25 °C and a working pressure of 200 kPa.

In general new membranes were used to determine the properties but some experiments were repeated with cleaned, used membranes. This was done to demonstrate that membranes change their properties with use.

1. The Effect of Ionic Strength on Pure Water Flux

Fig.11 illustrates the effect of ionic strength on pure water flux for a hollow fibre polysulphone membrane (CFP-2) and Fig.12 for a flat nylon membrane (Nypor^{TM}).

The flux of the polysulphone membrane increased from 230 $\ell/m^2/h$ to 330 $\ell/m^2/h$ when 0.05 M NaCℓ was added and to 400 $\ell/m^2/h$ when the salt concentration was doubled. Further addition of salt did not yield a higher flux. The corresponding result with a new membrane was an increase from 900 $\ell/m^2/h$ to 1400 $\ell/m^2/h$ in the presence of 0.1 M NaCℓ.

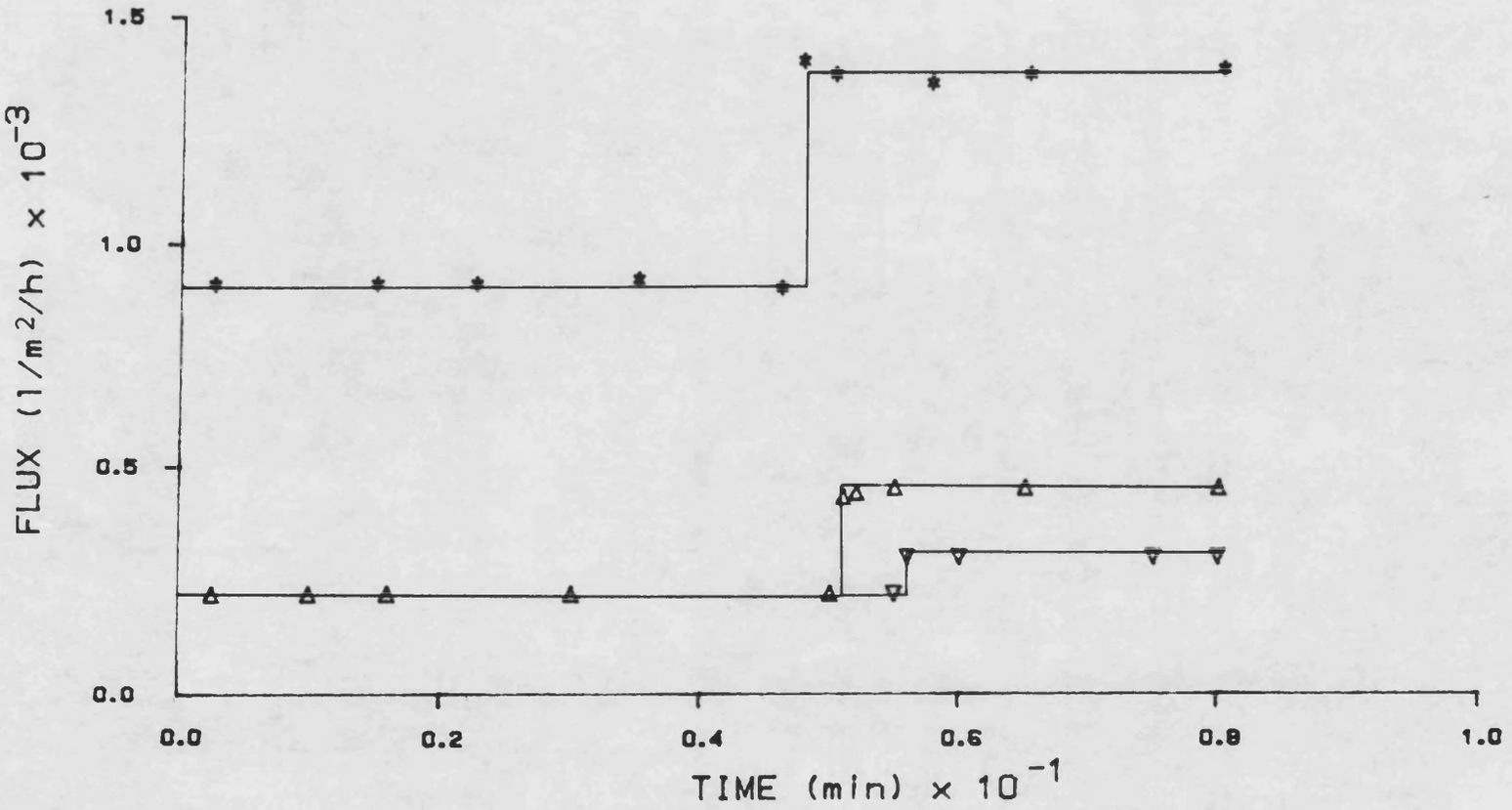


Fig.11: * CFP-2E-3 Membrane (new) Δ,∇ CFP-2D-3 Membrane (used, clean);
ΔP=20 kPa; Recycle Flow Rate=0.4 ℓ/min; T=10°C;
*, Δ 0.1 M NaCℓ; ∇ 0.05 M NaCℓ;

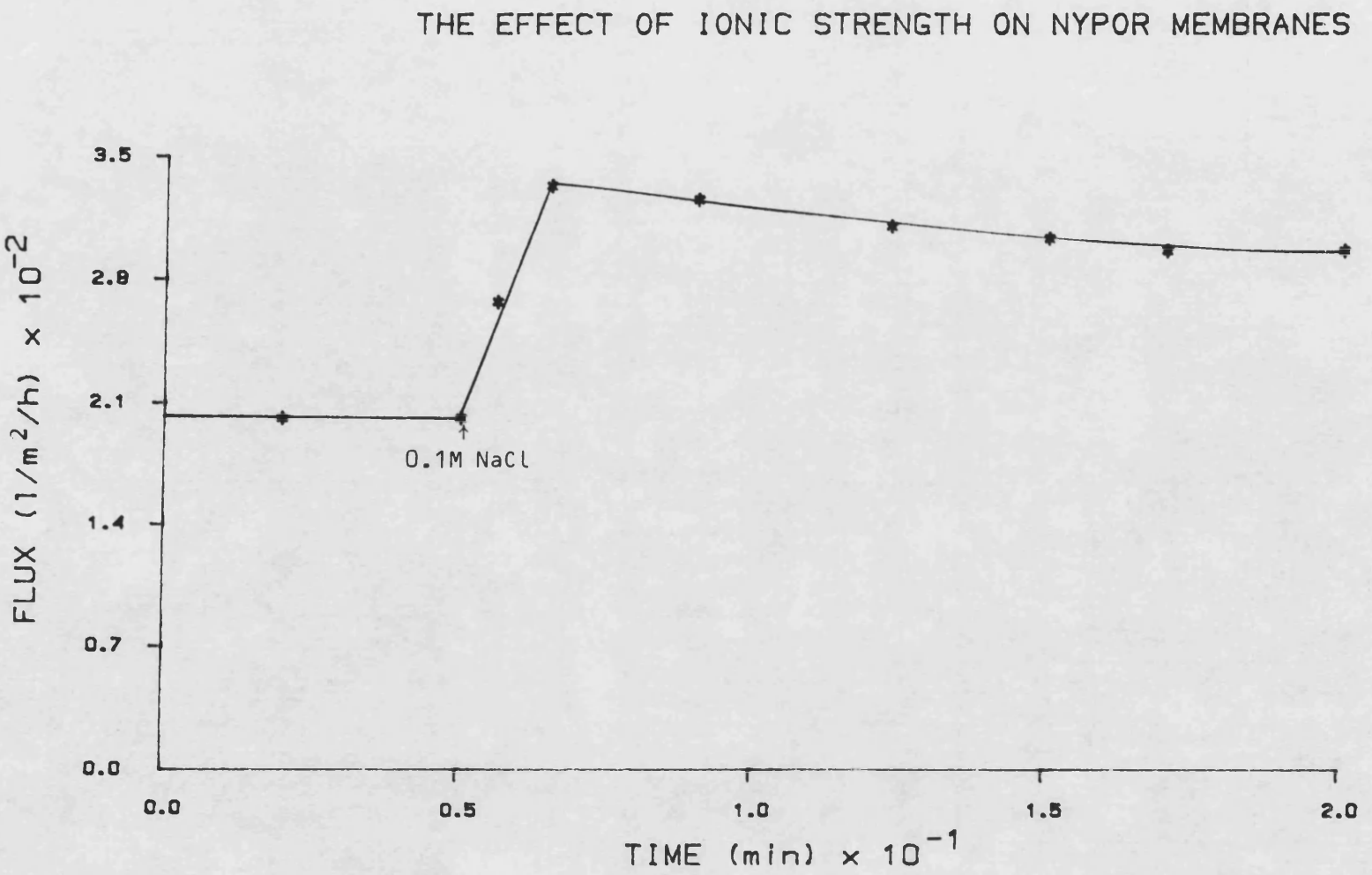


Fig.12: Nypor™ Membrane (0.2 μm);
ΔP=20 kPa; Recycle Flow Rate=0.5ℓ/min; T=10°C;

A qualitatively similar result was obtained for Nypor^{TM} membranes where a 50% flux increase was noted after the addition of 0.1 M NaCℓ.

Choe et al (1986) made the same observation when NaCℓ was present. They also noticed a flux increase of nearly the same ratio when they substituted $CuC\ell_2$ for NaCℓ but at a much lower concentration (Table 10).

TABLE 10 Effect of Salt on the Permeability of an IRIS 3042 Membrane (MW cut off: 20,000) (Choe et al 1986).

NaCl mol/ℓ	Flux ℓ/m²/h	$CuC\ell_2$ mol/ℓ	Flux ℓ/m²/h
0	287	0	272
0.05	314	0.001	317
0.15	345	0.002	325
0.5	349	0.005	329

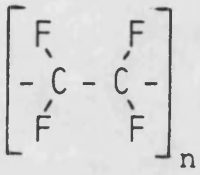
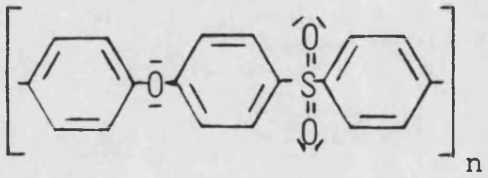
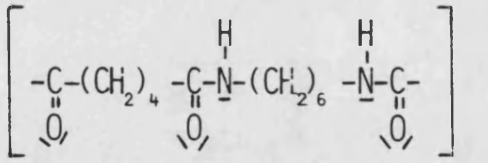
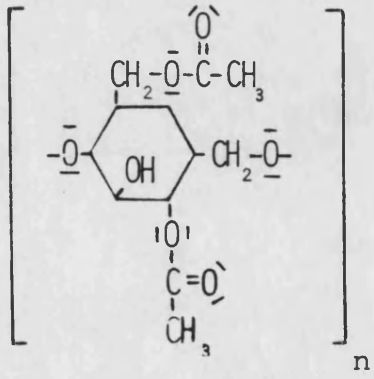
Choe et al declined to speculate on an explanation, but electrostatic interactions between the pore and ions in solution could have an effect. The surface of organic polymers immersed in an aqueous medium is charged, usually negatively (Norde 1981). This attracts a layer of oppositely charged ions from the solution and a diffuse double layer of counter-ions, whose radius of electrical influence reduces the effective pore size. The negatively charged surface is embedded by a positively charged double layer. As most ions of the reverse osmosis treated water at pH 5.5 are also positively charged passage through the membrane is likely to be restricted by the electrostatic effects of the double layer. Addition of salt compresses the diffuse double layer hence the radius of electrical influence and thus eases passage of fluid. This effect is 50-100 times more pronounced with doubly charged ions and hence causes the same effect at much lower salt concentrations as observed.

2. The Effect of pH on Pure Water Flux

As with the addition of salt, changing the pH of the feed resulted in an altered membrane permeability as demonstrated in Fig.13.

The flux reduction at higher pH observed with a new polysulphone membrane was only minor compared to the flux reduction of the same membrane after exposure to cheese whey followed by cleaning. The used membrane showed a flux decline already around pH 4 and the relative change in flux was more pronounced than with a new membrane.

TABLE 11 Chemical composition of membranes

Membrane	Material	Composition
Gore-Tex	PTFE	
CFP-2	Polysulphone	
Nypor™	Polyamide	
Asypor™	Cellulose Acetate	

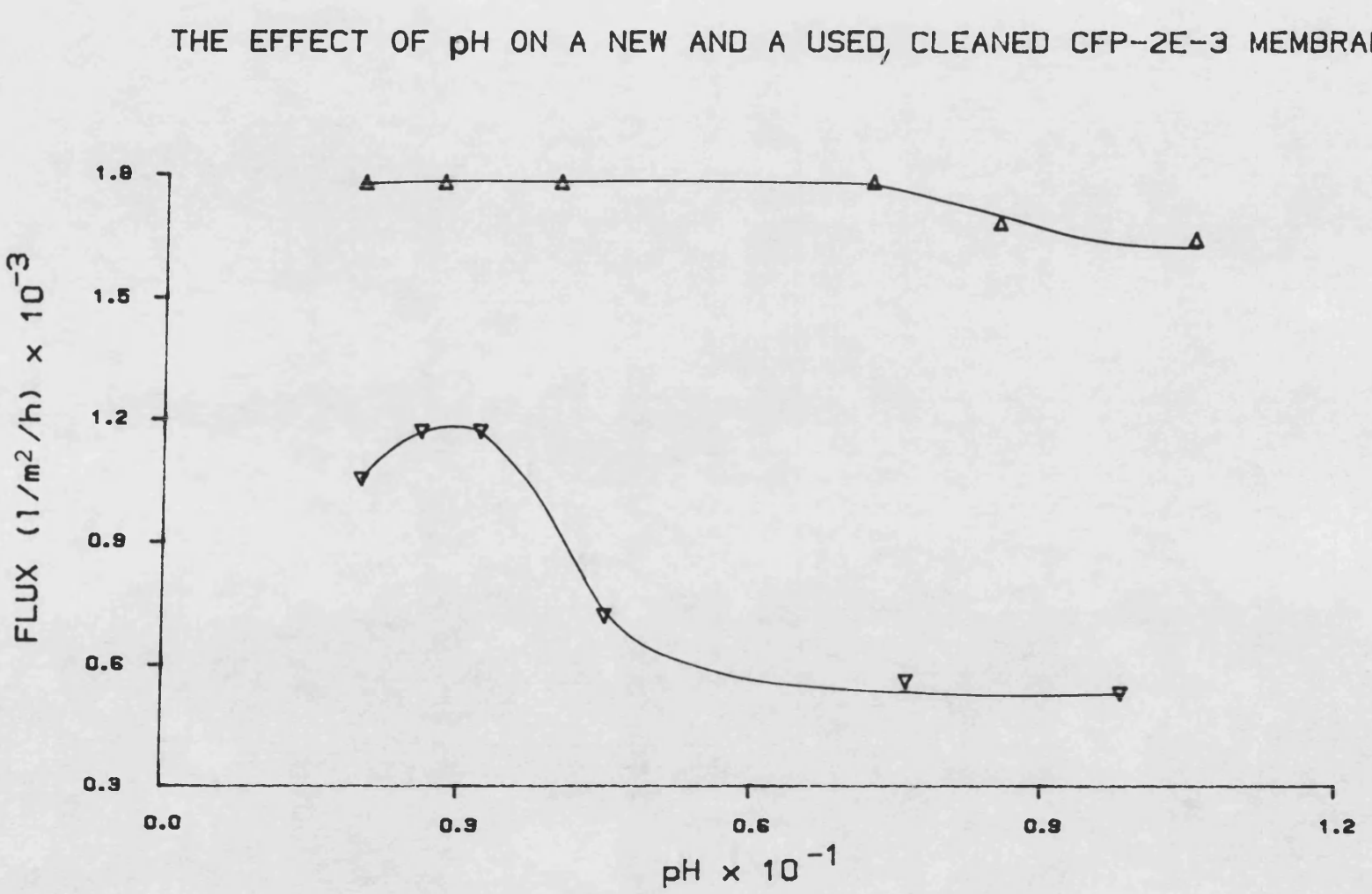


Fig.13: CFP-2E-3 Membrane; ΔP=20 KPa; Recycle Flow Rate=0.4ℓ/min; T=10°C;
Δ new membrane; ∇ used, cleaned membrane;

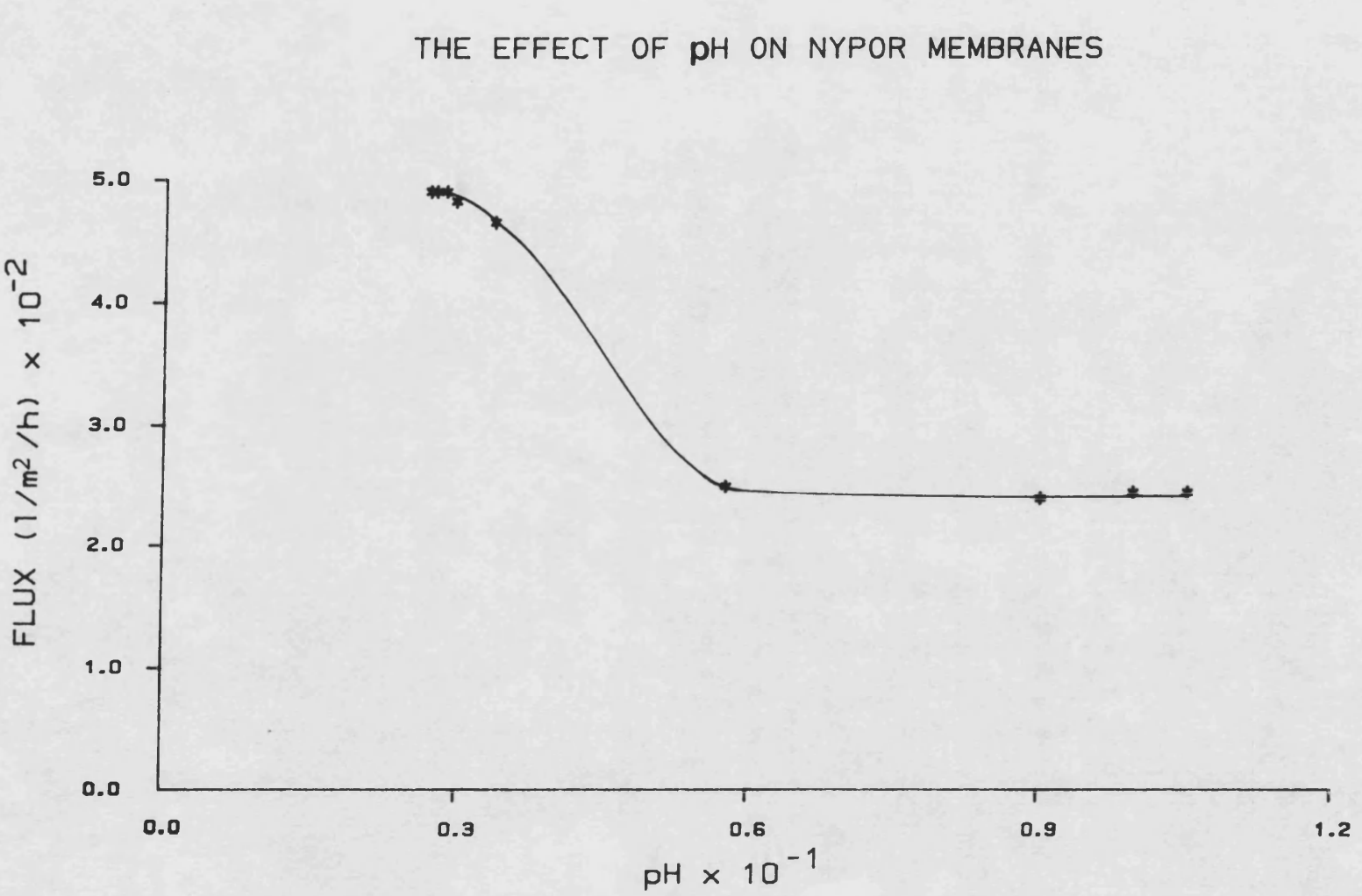


Fig.14: Nypor™ Membrane;
ΔP=20 KPa; Recycle Flow Rate=0.5ℓ/min; T=10°C;

Provided the diffuse double layer induces a positively charged membrane surface one would expect a higher flux at higher pH, since the charge barrier is absent but the opposite was observed, suggesting a potentially determined surface. A possible explanation could be found in the composition of the membrane. It is made of an organic polymer which possesses functional groups in sequential repetition. Table 11 lists the composition of the membranes used in this work. Presumably at a pH considerably higher than the neutral point the sulphone groups of the CFP-2 membrane are negatively polarised and perhaps even ionised at extreme values of pH. Similar conjectures were made by Sivik and Matthiasson (1980). The negative polarisation is unlikely to be entirely balanced by counter-ions. Therefore, water at a high pH is likely to be repelled.

Fig.13 demonstrates distinctly the changed properties of membranes once exposed to a proteinaceous solution. The flux decline is shifted towards a lower pH. This behaviour is not attributable to the membrane any more but reflects the properties of the "second membrane", the adsorbed fouling layer. Knowing from the membrane history that it was previously exposed to whey proteins and that the permeability in the presence of this particular protein mixture was highest at low pH, it is strongly indicated that the characteristics of the adsorbed protein layer are reflected rather than that of the membrane itself.

The effect of pH on a membrane made of nylon is stronger than the effect on a polysulphone membrane as shown in Fig.14. It appears that the amide groups polarise more strongly than the sulphone groups where the pH dependency was less pronounced. The assumption seems to be reasonable since the nitrogen atom of the amide group exhibits a negative inductive influence and stabilises the negative centre around the oxygen atom. The amide group is therefore more polarised than the sulphone group and produces a stronger charge barrier, hence, water flux is more reduced.

3. The Effect of Temperature on Flux

Fig.15 shows that the flux increases at lower temperatures and levels off at higher temperatures. The initial increase in flux is a result of decreasing viscosity with increasing temperatures and presumably also because of the weaker charge effects at higher temperatures. Reduction of pore size due to longitudinal expansion in the pores could be the reason for the levelling off of flux at very high temperatures.

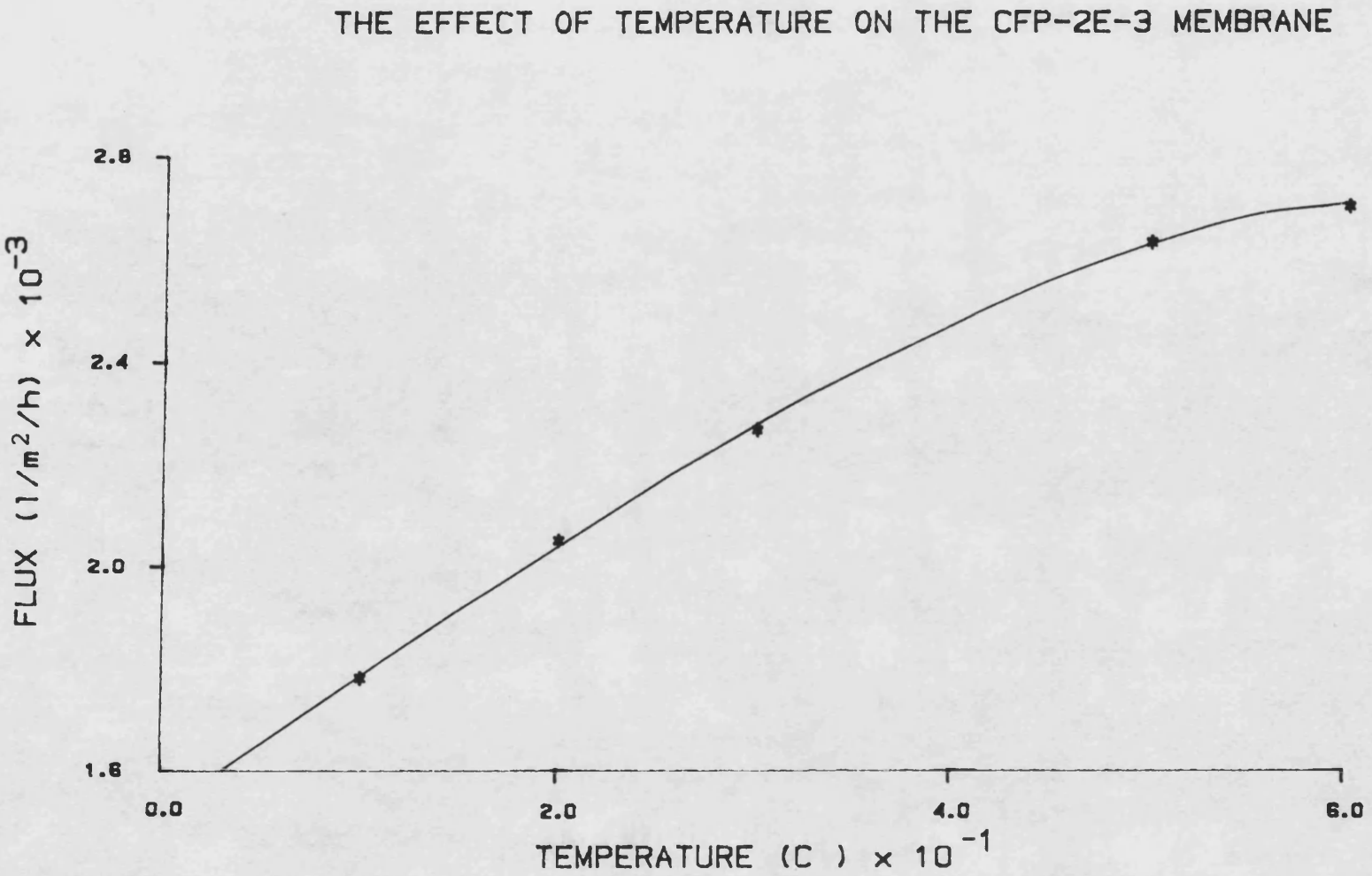


Fig.15: CFP-2E-3 Membrane;
ΔP=20 kPa; Recycle Flow Rate=0.4ℓ/min;

Part II

Membrane Fouling by Proteins

The observations in part I of altered membrane characteristics with changing pH and ionic strength indicate strongly the need to investigate the permeability and rejection properties of membranes when exposed to proteinaceous solutions under various environmental conditions. Thus the fouling effect of different single protein and mixed protein systems on membranes under various experimental conditions such as pH and ionic strength was investigated.

1. Single Protein Systems

This section is divided by protein species for ease of presentation.

1.1 BSA

CFP-2 membranes were used to filter a solution of bovine serum albumin in order to investigate transmembrane permeability and rejection behaviour at different pH.

Figs.16 and 17 illustrate the protein transmission and the flux profile for a 1.5 g/ℓ BSA solution at pH 2.8, 4.7 and 9.

Protein transmitted at a low level initially, then increased passing through a maximum before steadily declining. In cases of a pH different from the Iso electric point (I.E.P ~ 5) transmission after having passed through the maximum declined significantly before levelling off into a steady state transmission. The decline phase was absent or distinctly less pronounced for BSA solutions adjusted to the I.E.P. Furthermore the steady state transmission was 70%, whereas it was only 55% and 35% at pH 9 and pH 2.8 respectively.

The transmembrane permeability showed a reverse response with respect to pH. The flux of solutions at a pH away from the I.E.P was in general higher when compared to the flux of a BSA solution around pH 5.

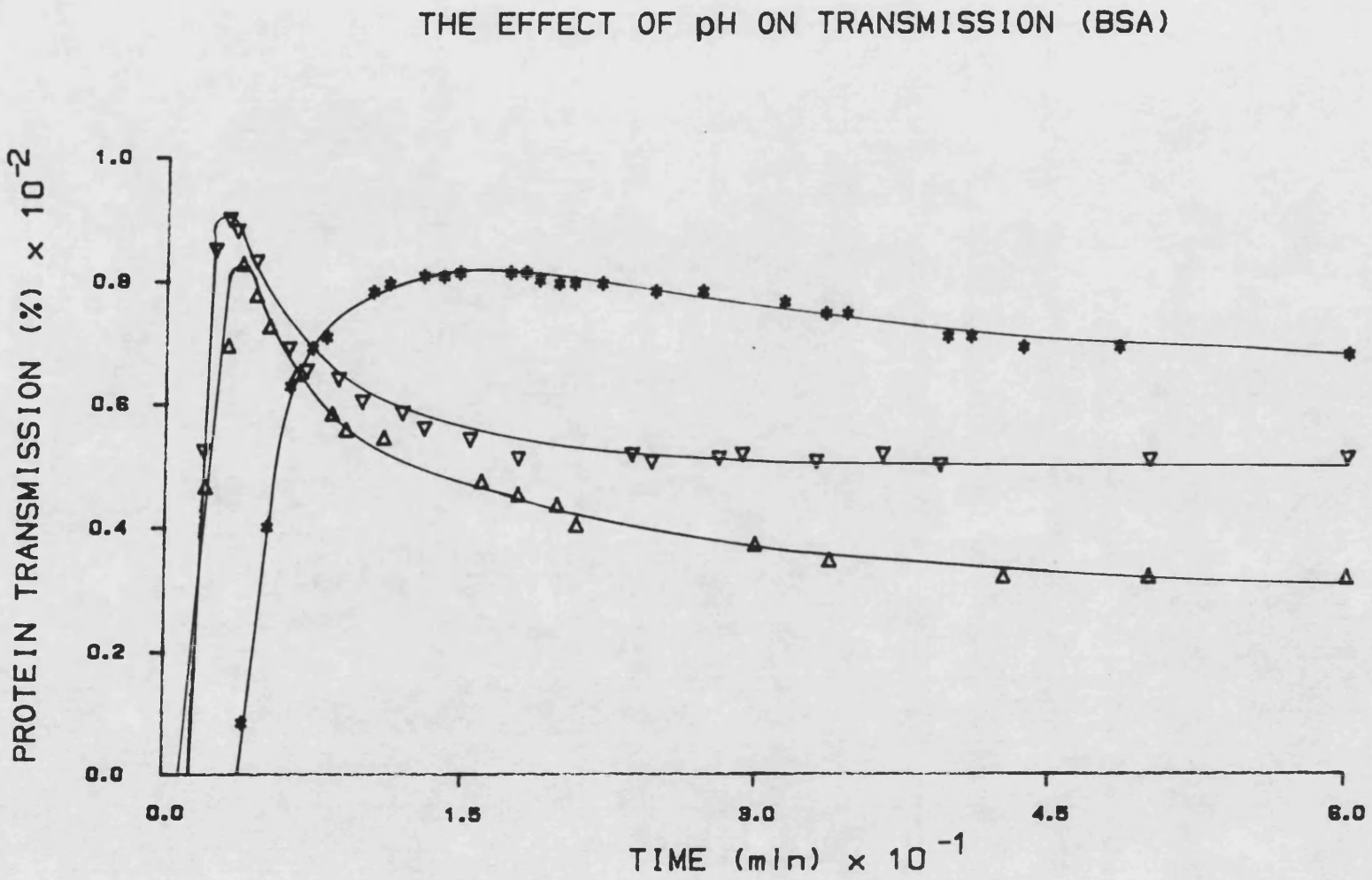


Fig.16: CFP-2E-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: BSA; C_b=1.5 g/ℓ; Δ pH=2.8; * pH=4.7; ∇ pH=9;

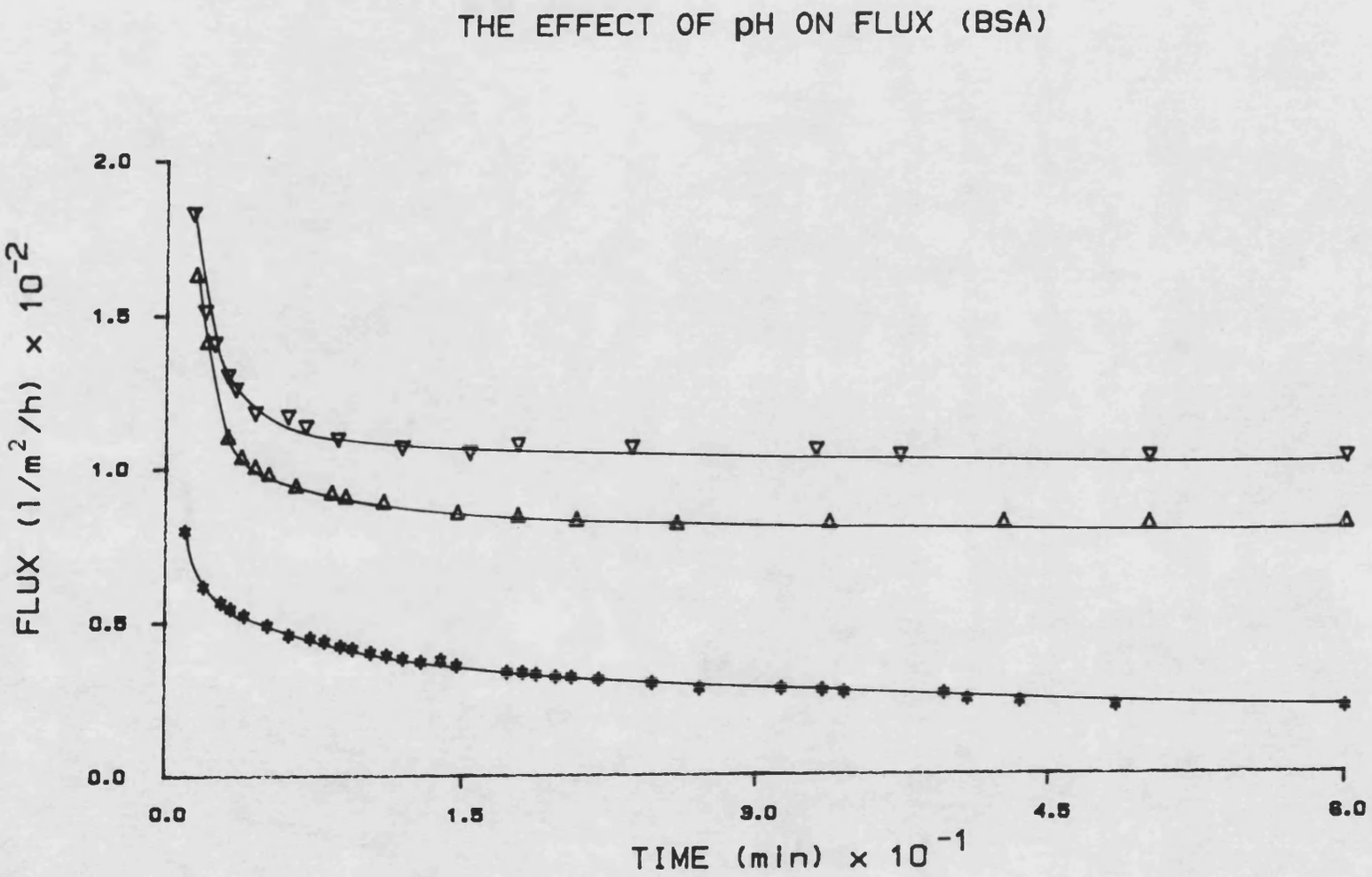


Fig.17: CFP-2E-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: BSA; C_b=1.5 g/ℓ; Δ pH=2.8; * pH=4.7; ∇ pH=9;

1.2 Ovalbumin

Ovalbumin (4 g/ℓ) was used to show that the pH and ionic strength as well as the type of salt affect the protein transmission and flux.

Fig.18 demonstrates that the addition of salt enhanced the steady state transmission considerably and reduced the degree of transmission loss. In the absence of salt transmission was only 40% whereas NaCℓ increased transmission to 65% and potassium phosphate to 75%. On the other hand transmembrane permeability was highest in the salt-free case and distinctly reduced when NaCℓ or potassium phosphate was added (Fig.19). Figs.20 and 21 show the effect of pH on the steady state values of protein transmission and flux in the absence and presence of salt. The results are in good agreement with the observations made with BSA. Protein transmission was highest around the I.E.P (~4.6) and clearly reduced at lower and higher values of pH. In the presence of salt the overall transmission was enhanced except around pH 4.6. Membrane permeability was lowest around the I.E.P and in the presence of salt. It is notable that NaCℓ has a stronger influence in reducing flux than potassium phosphate.

1.3 Casein

A CFP-2 polysulphone membrane was used to look at the fouling effect of casein at different values of pH.

Casein is a rather big protein (MW ~ 120,000) and only sparingly soluble in water. Hence, it must be considered as a colloidal suspension rather than a solution.

Fig.22 and 23 show the steady state protein transmission and flux profile over a range of pH with no salt being present. In contrast to the previous observations, rejection was lowest around the I.E.P. Casein transmission exhibited a minimum at pH 4.6. The flux varied by less than a factor of two over the whole range of pH.

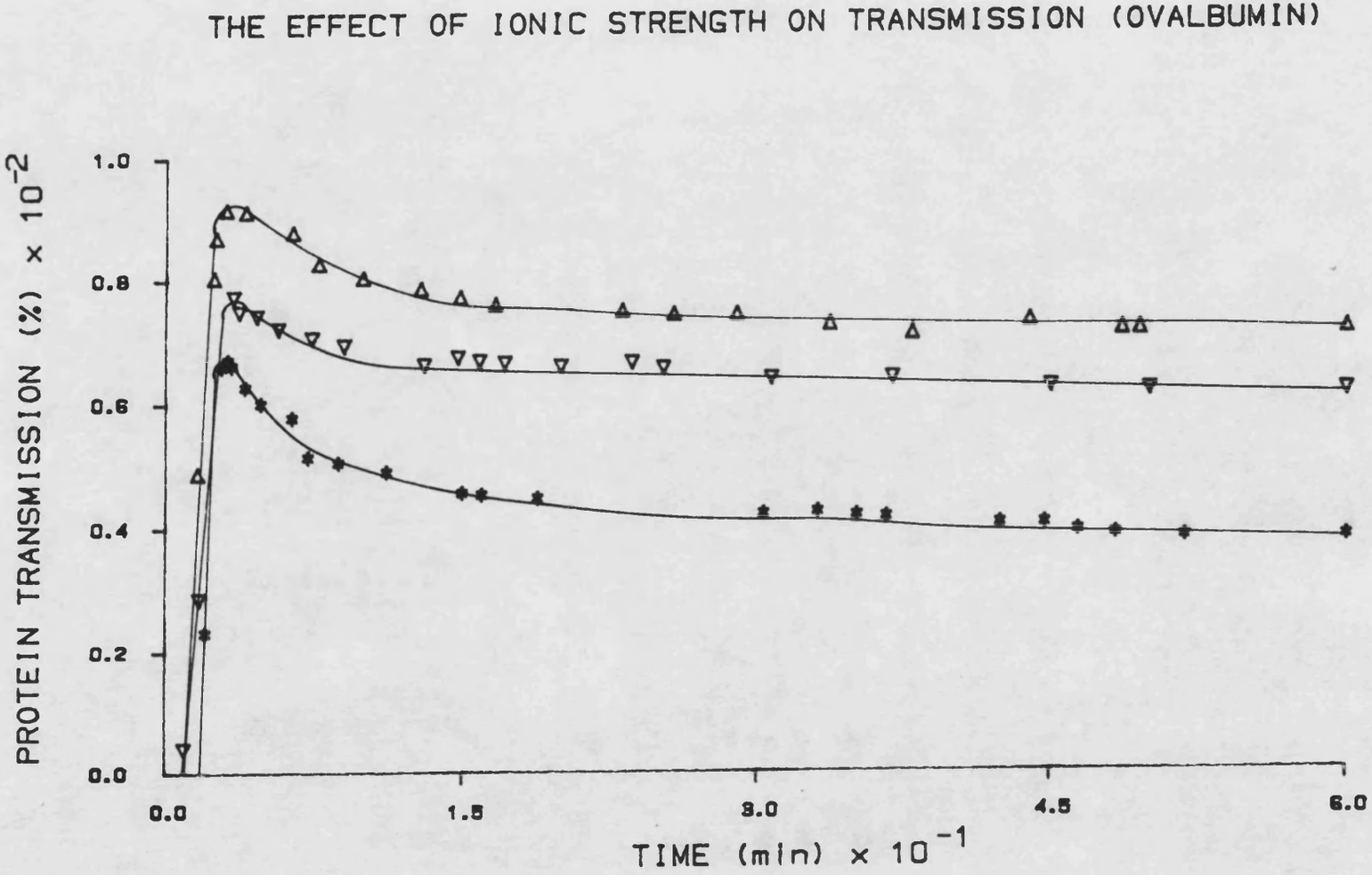


Fig.18: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Ovalbumin; C_b=4 g/ℓ; pH=9.2;
Δ 0.1 M Potassium Phosphate; ∇ 0.1 M NaCℓ; * no salt;

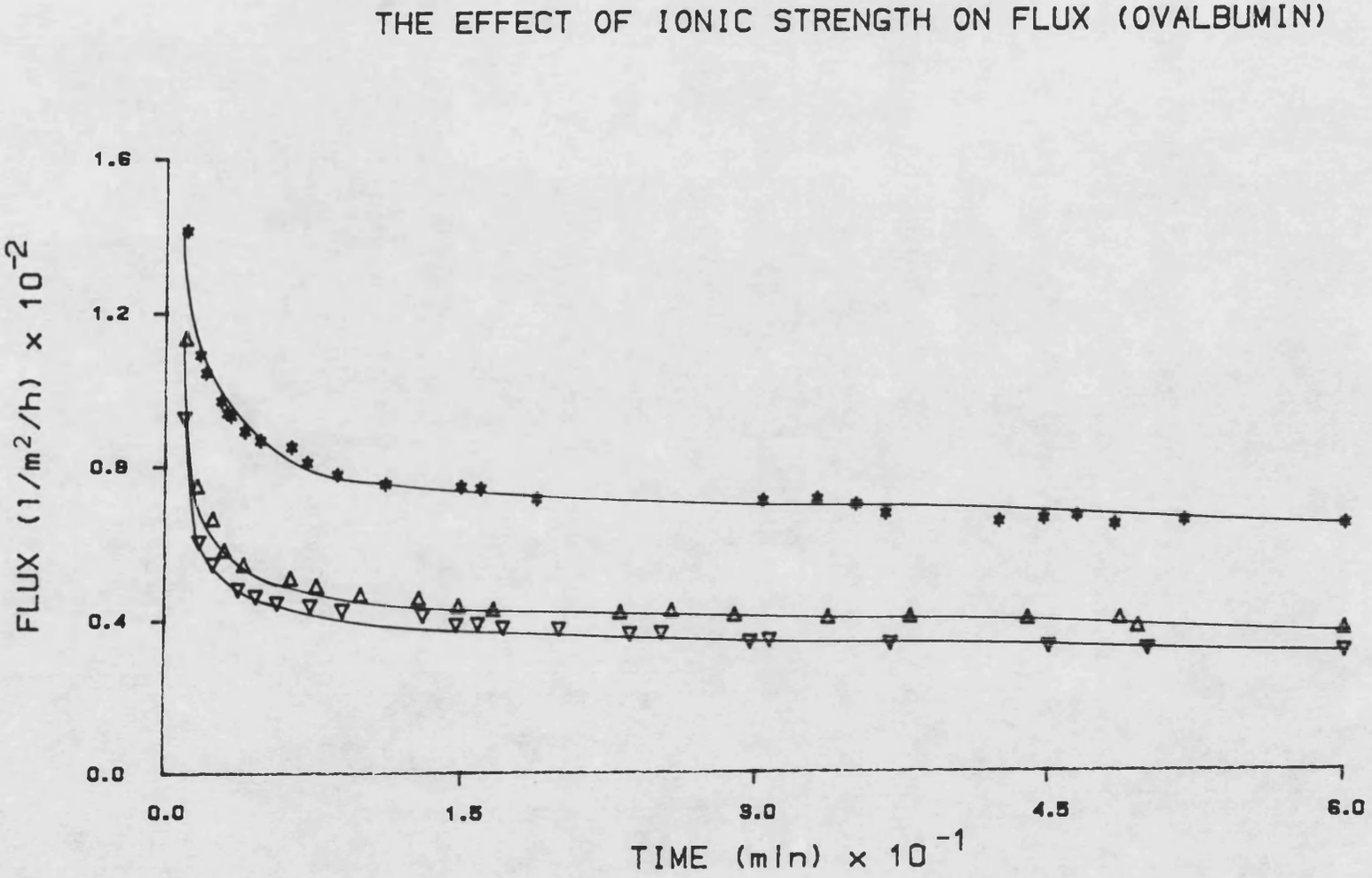


Fig.19: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Ovalbumin; C_b=4 g/ℓ; pH=9.2;
Δ 0.1 M Potassium Phosphate; ∇ 0.1 M NaCℓ; * no salt;

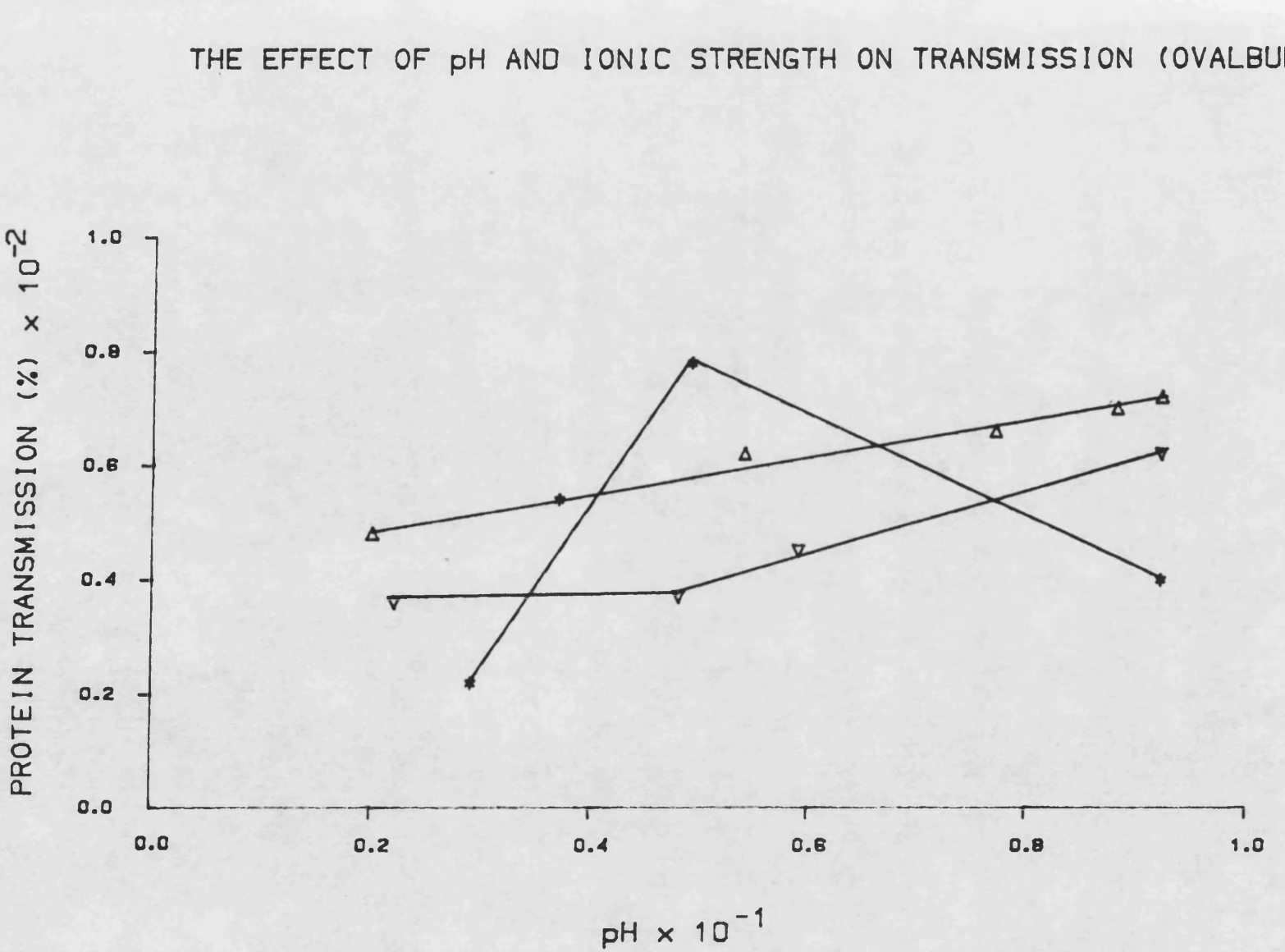


Fig.20: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Ovalbumin; C_b=4 g/ℓ;
▽ 0.1 M NaCℓ; * no salt; Δ 0.1 M Potassium Phosphate;

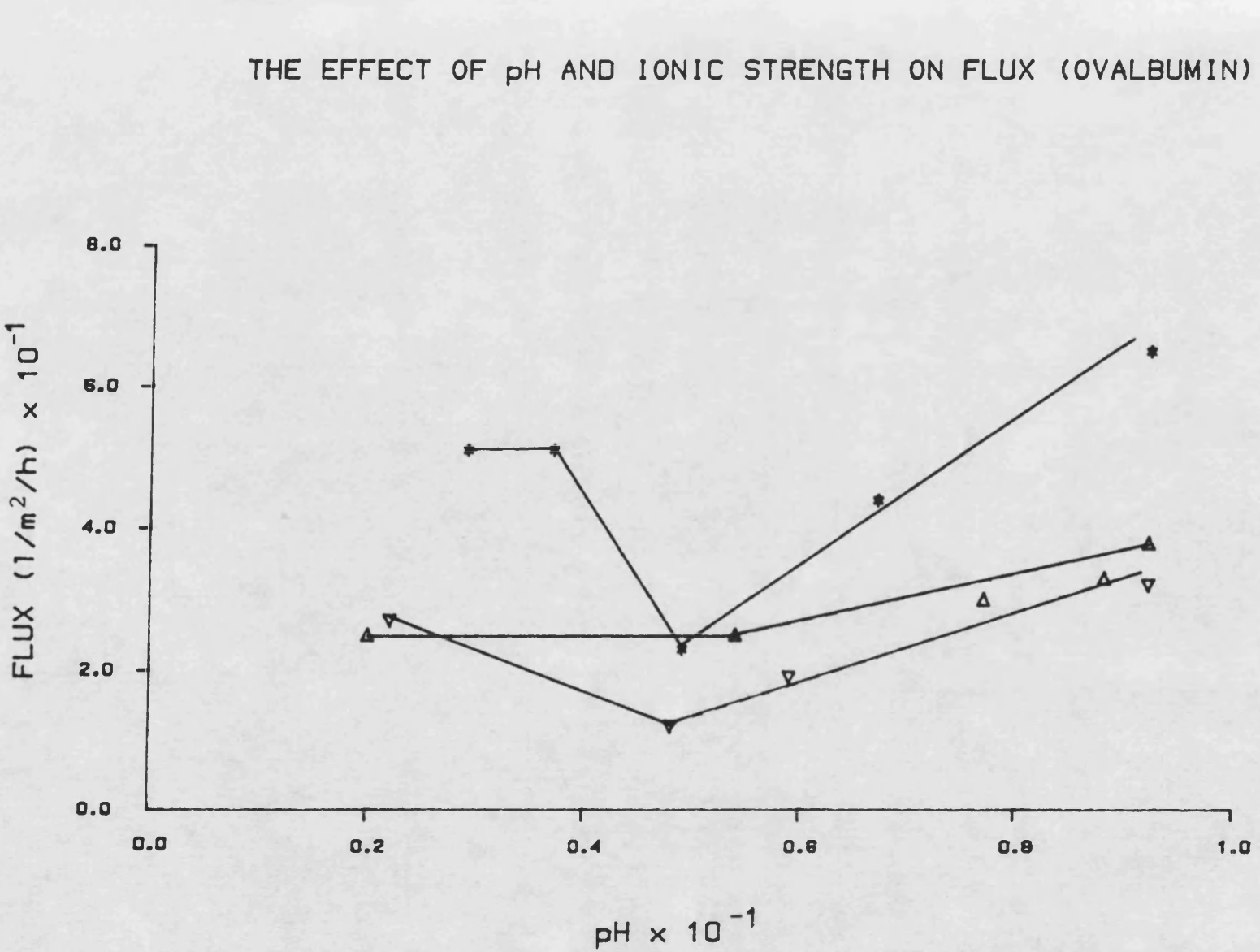


Fig.21: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 κPa; T=10°C;
Feed: Ovalbumin; C_b=4 g/ℓ;
▽ 0.1 M NaCℓ; * no salt; Δ 0.1 M Potassium Phosphate;

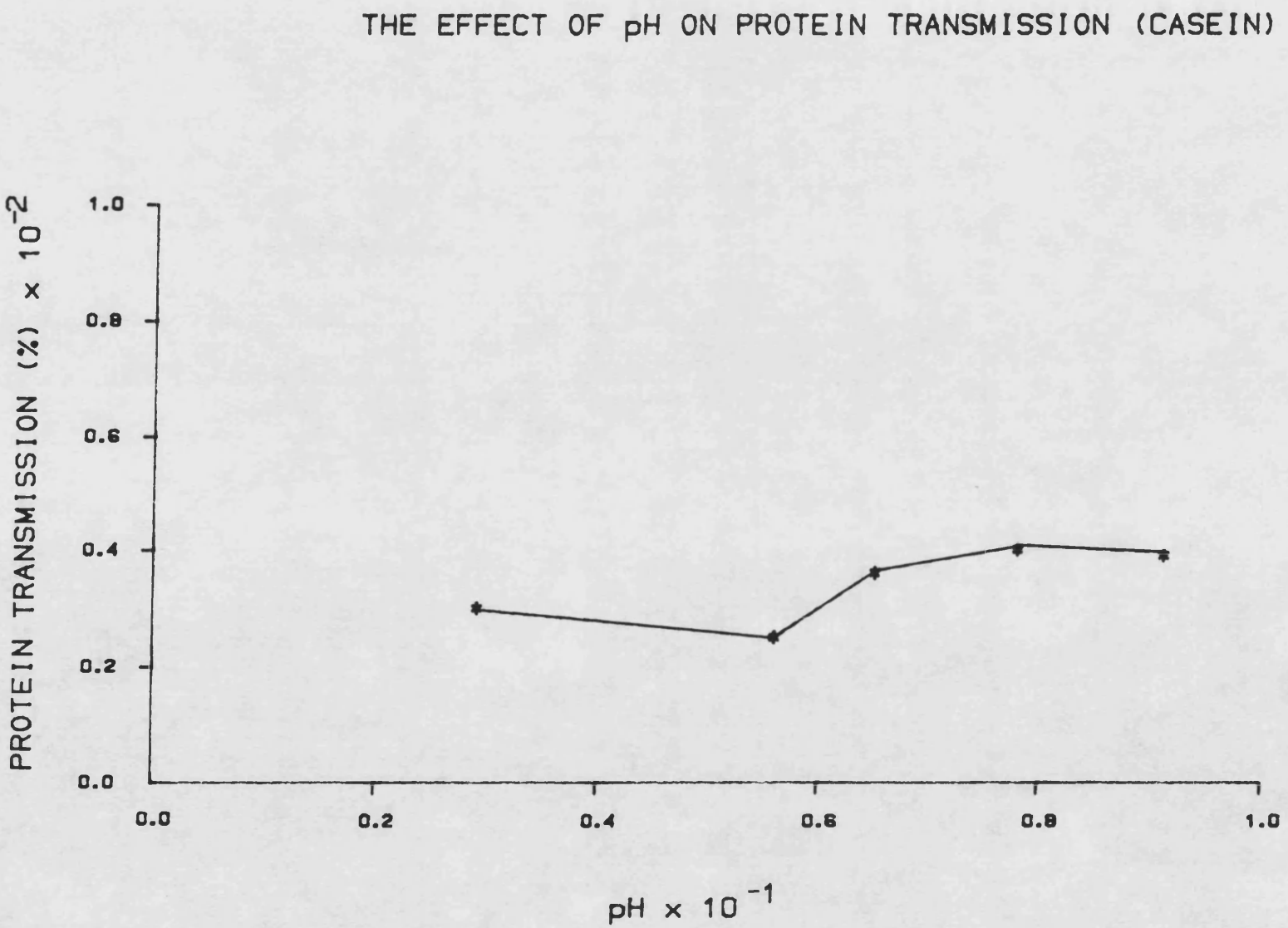


Fig.22: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Casein; C_b=3.3 g/ℓ; OSU (Open Side Up Configuration);

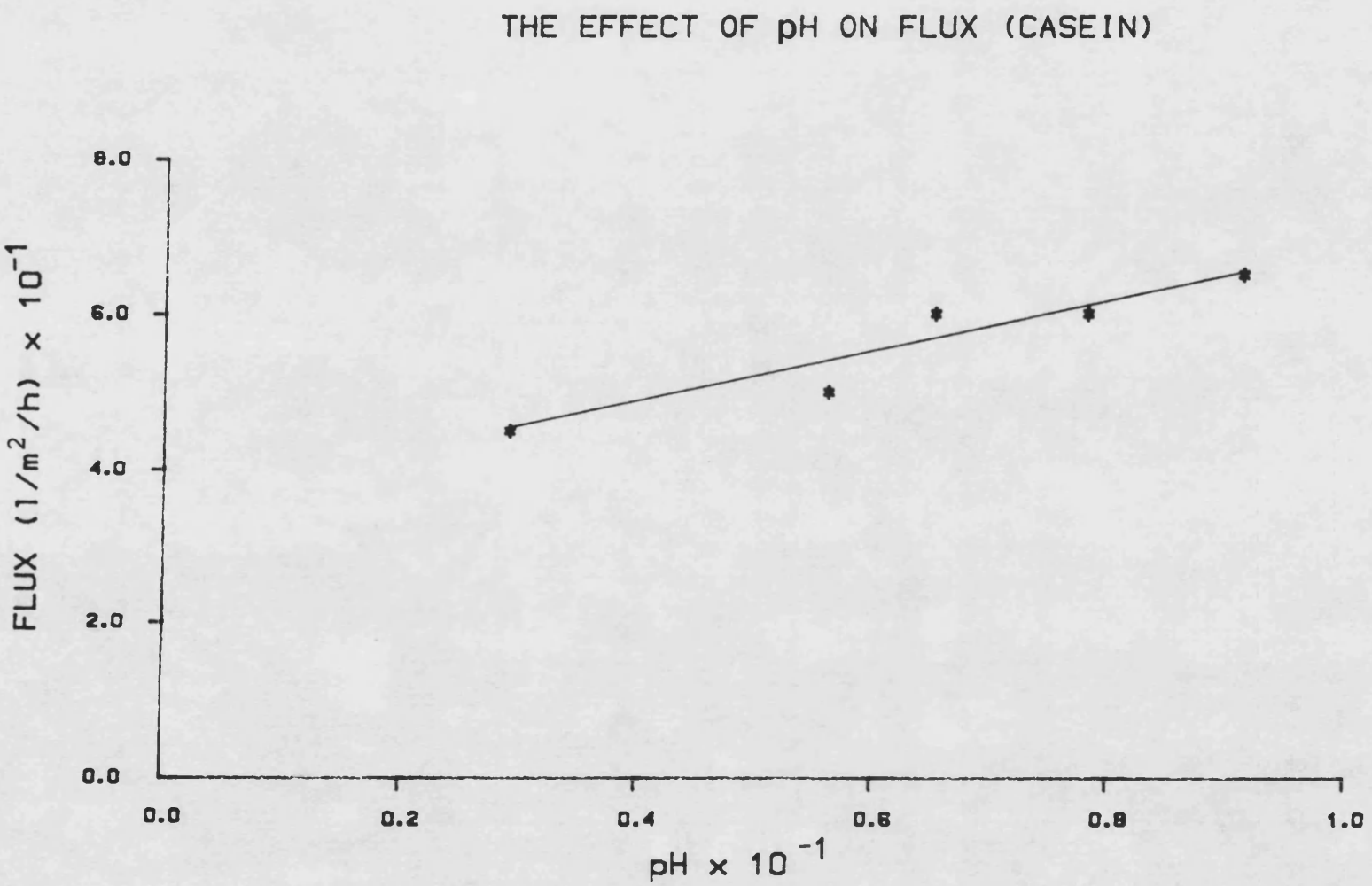


Fig.23: CFP-2D-3 Membrane; Recycle Flow Rate=0.9 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Casein; C_b=3.3 g/ℓ; OSU;

1.4 Pepsin

The purpose of this experiment was to investigate the fouling effect of a protein with an almost constant charge over a wide range of pH. A CFP-2 membrane was used to separate pepsin. Pepsin is a hydrolytic active enzyme with an I.E.P < 2. It is therefore negatively charged over the range of pH applied.

Fig.24 shows that protein transmission with 32% was constant at pH 3-10 and significantly reduced with 23% at pH 2.5. The increased transmission at pH > 10 was presumably due to denaturation of pepsin. Unexpected were the flux variations at pH 3-7 (Fig.25). As the protein transmission over this range of pH was not altered because of electrical changes flux also should remain unaffected. Transmembrane permeability around the I.E.P was distinctly reduced which is in agreement with previous observations. The unusually high overall flux of 290 $\ell/m^2/h$, with minor signs of long-term fouling (Fig.26), is remarkable.

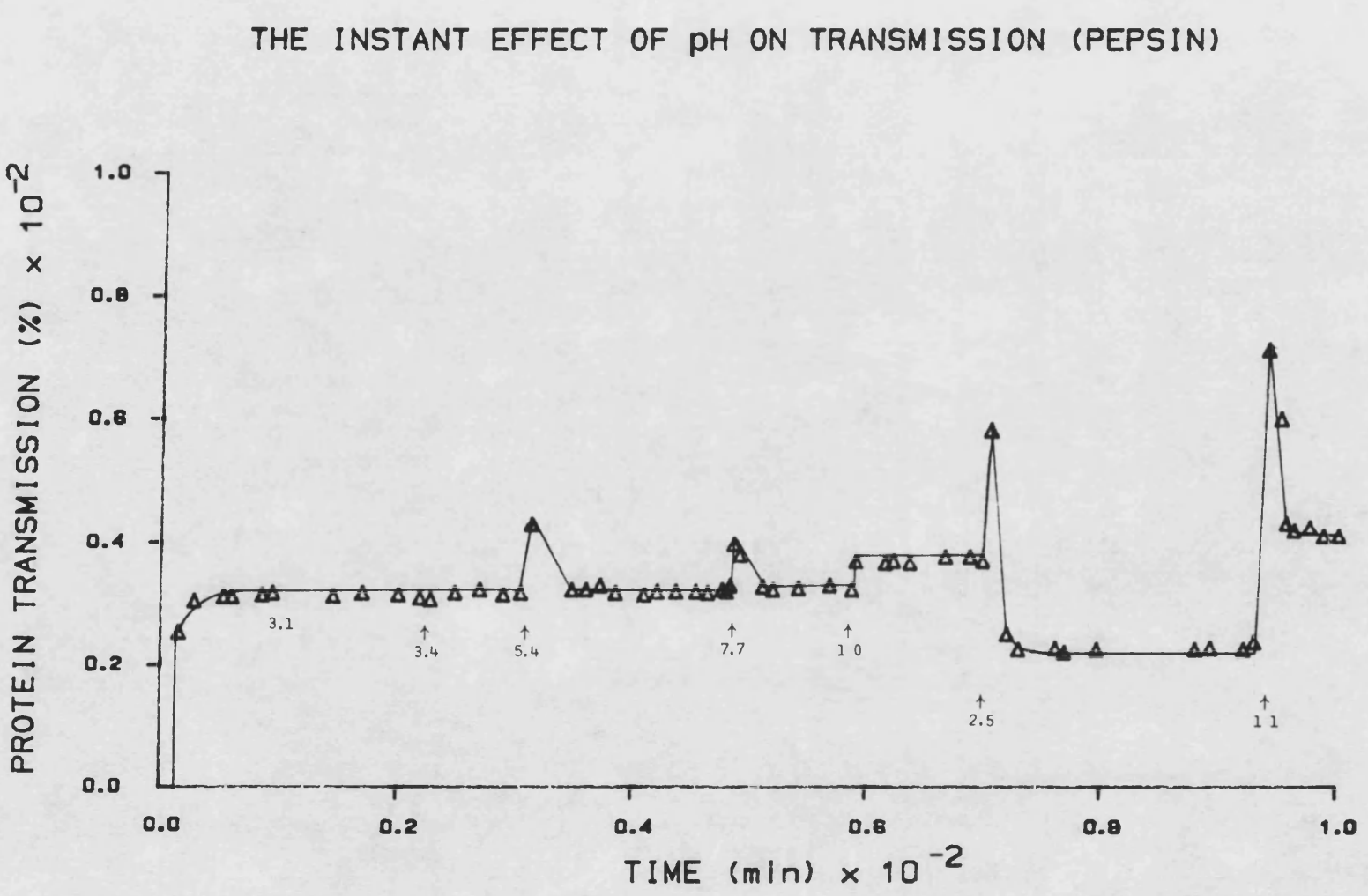


Fig.24: CFP-2D-3 Membrane; Recycle Flow Rate=1 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Pepsin; C_b=5 g/ℓ; OSU; pH as shown

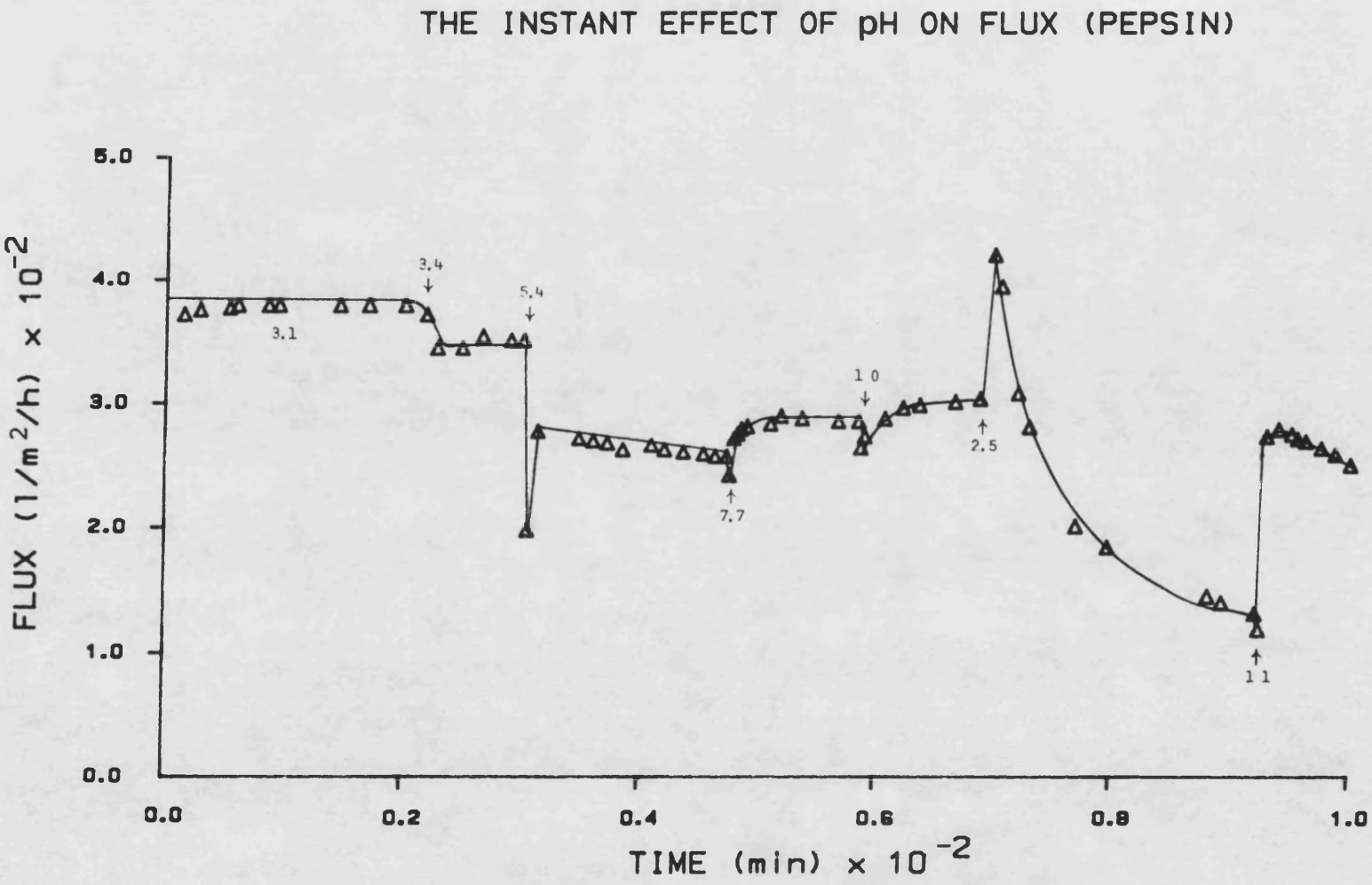


Fig.25: CFP-2D-3 Membrane; Recycle Flow Rate=1 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Pepsin; C_b=5 g/ℓ; OSU; pH as shown

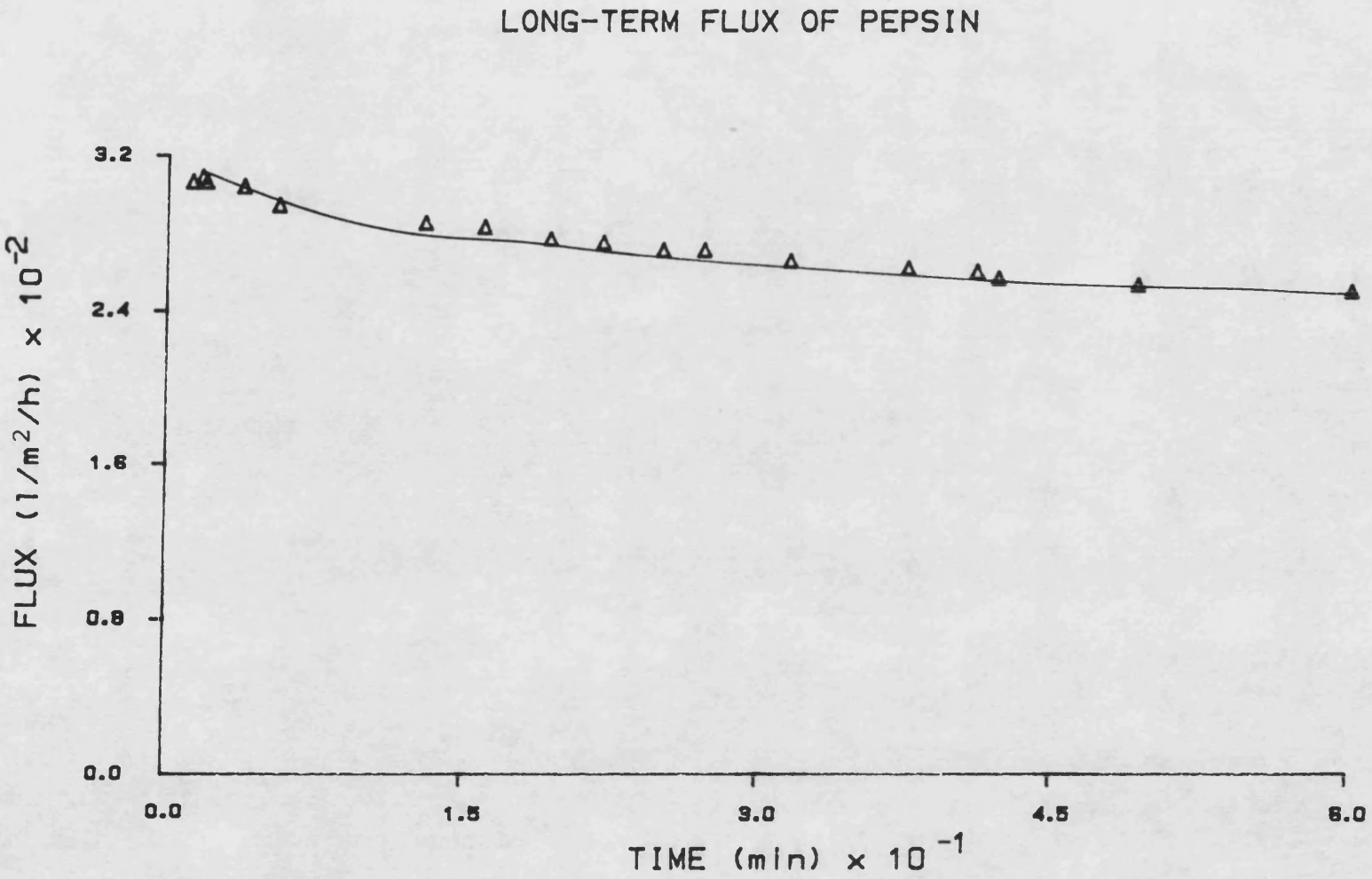


Fig.26: CFP-2D-3 Membrane; Recycle Flow Rate=1 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Pepsin; C_b=5 g/ℓ; pH=7; OSU;

2. Mixed Protein Systems

2.1 Cheese Whey Eluate and Concentrate

CFP-2 membranes were used to investigate the fouling effect of a mixed protein system with respect to pH and ionic strength. The transmission profile of a low concentration whey protein solution (1.5 g/ℓ) at different pH for a nearly salt-free case and in the presence of 0.1 M NaCℓ is shown in Figs.27 and 28. Figs.29 and 30 demonstrate the effect on the environmental conditions described above when the solution applied of 14 g/ℓ had a high protein concentration.

Protein transmission of a mixed protein system also showed an initial increase to a maximum before declining thereafter and levelling off into a steady state. At none of the feed concentrations was there any subsequent decrease in transmission following the maximum when the solutions were adjusted to the I.E.P (4.2-5.2). At a protein concentration of 1.5 g/ℓ the overall transmission around the I.E.P was by far the highest with 80%. The steady state values at pH 3.5 and 8.5 were 55% and 48% respectively. Supplementary NaCl increased the steady state transmission to 30-35% at a pH away from the I.E.P and reduced the degree of loss in transmission. On the other hand a higher ionic strength caused an increasing rejection around the I.E.P.

The difference in transmission over the range of pH at a feed concentration of 14 g/ℓ is less distinct. However, the steady state transmissions with 60% and 55% at pH 3.5 and 8.5 are lower compared to 65% at the I.E.P. Adding salt to a high concentration protein solution did not yield a beneficial effect on protein transmission. It remained around 60%.

Transmembrane permeability of the low concentration protein solution was highest at pH 3.5 and lowest around the Iso electric point (Fig.31). In the case where NaCℓ was present (Fig.32) flux was significantly reduced to around 30 $ℓ/m^2/h$ over the range of pH applied.

Most striking were the results obtained when the feed was of a high protein concentration whilst the flux of a nearly salt-free protein solution was only around 10 $ℓ/m^2/h$ regardless of the pH (Fig.33); adding salt almost doubled the flux (Fig.34) except when the solution was adjusted to the I.E.P.

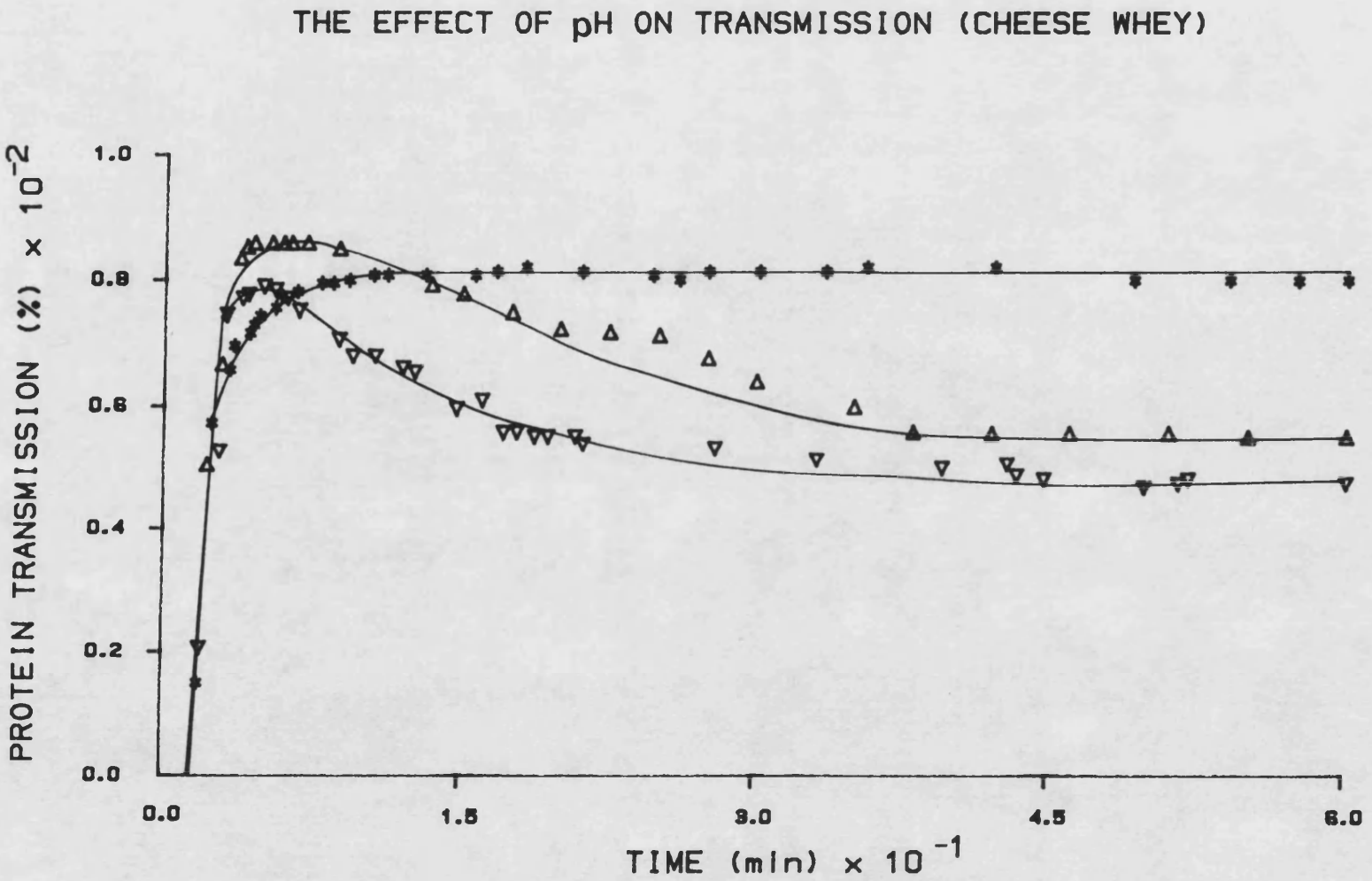


Fig.27: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein; C_b=1.5 g/ℓ; Δ pH=3.5; * pH=5.2; ∇ pH=8.5;

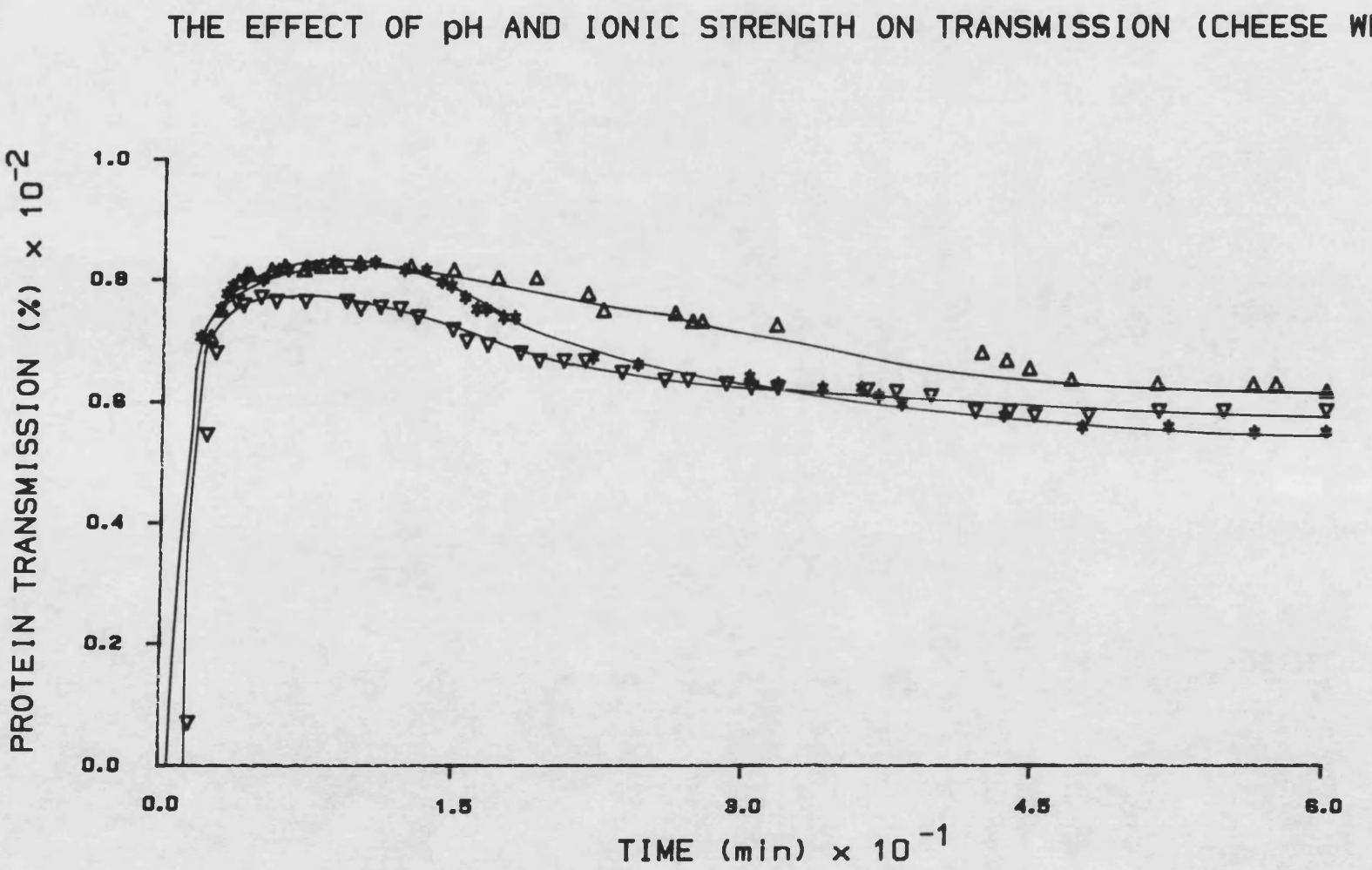


Fig.28: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein, C_h=1.5 g/ℓ; 0.1 M NaCℓ;
∇ pH=8.5; Δ pH=3.5; * pH=5.2;

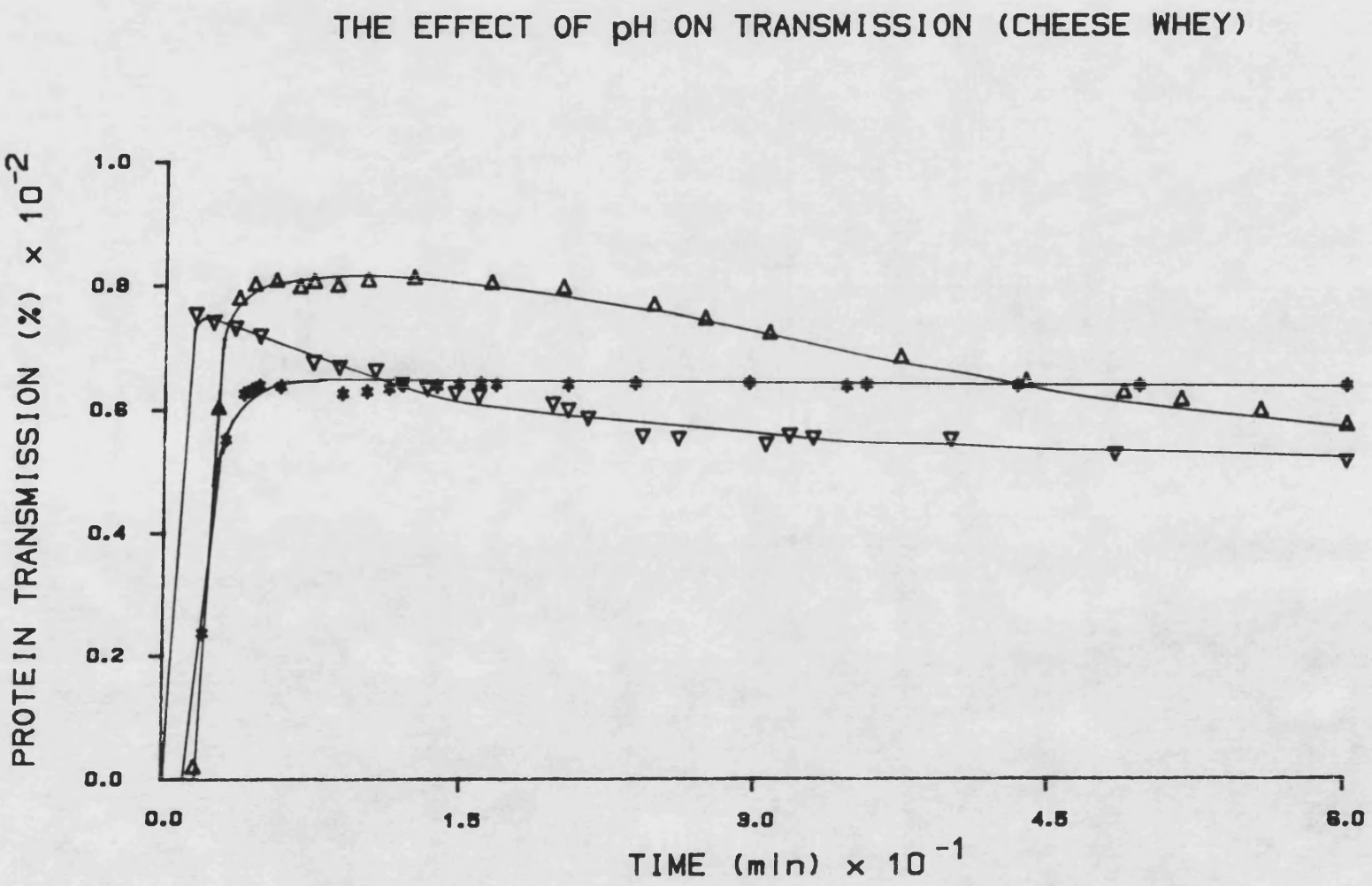


Fig.29: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein, C_b=13.4 g/ℓ; Δ pH=3.5; * pH=4.2; ∇ pH=8.5;

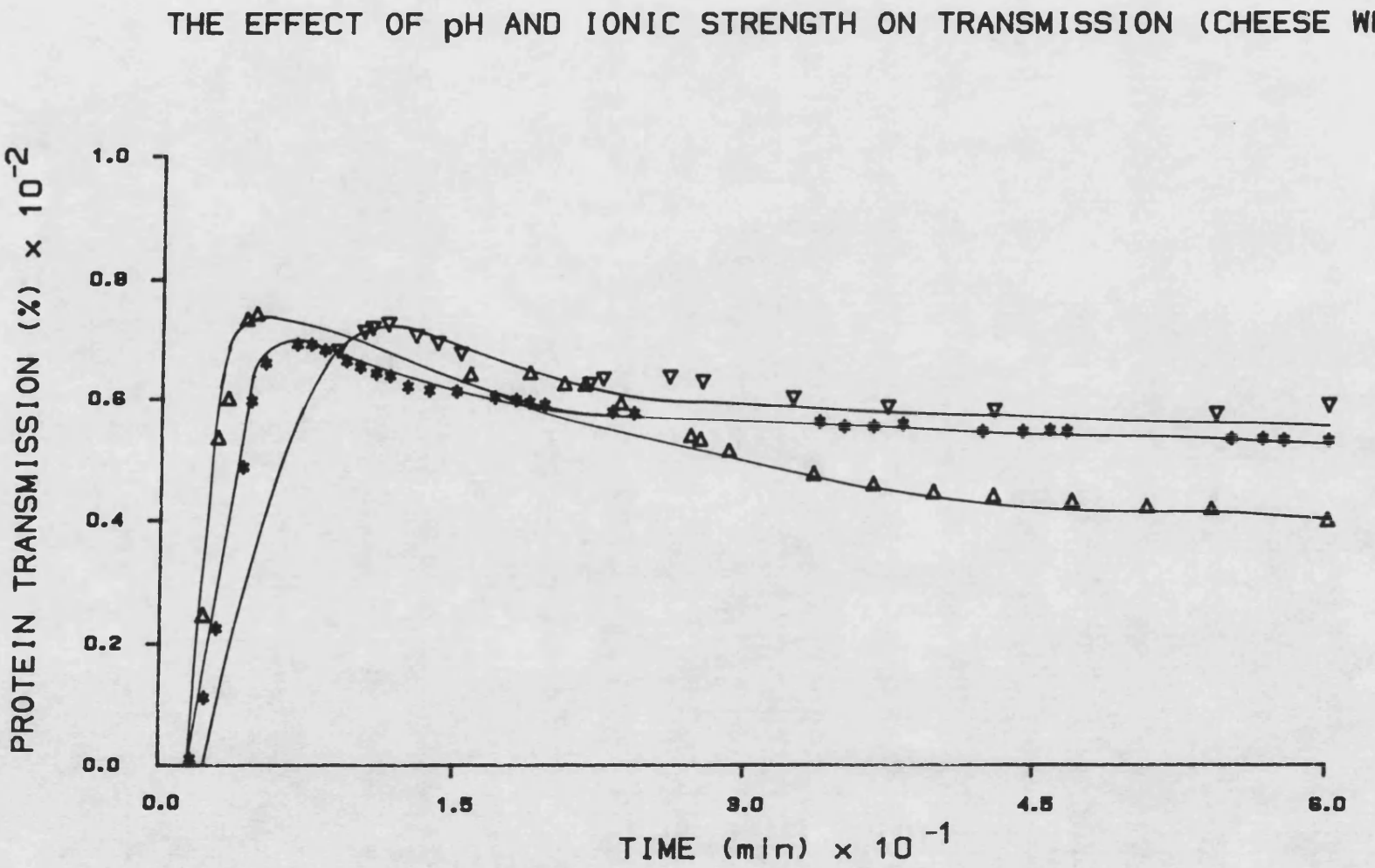


Fig.30: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein; C_b=13.0 g/ℓ; 0.1 M NaCℓ;
Δ pH=3.5; * pH=4.2; ∇ pH=8.5;

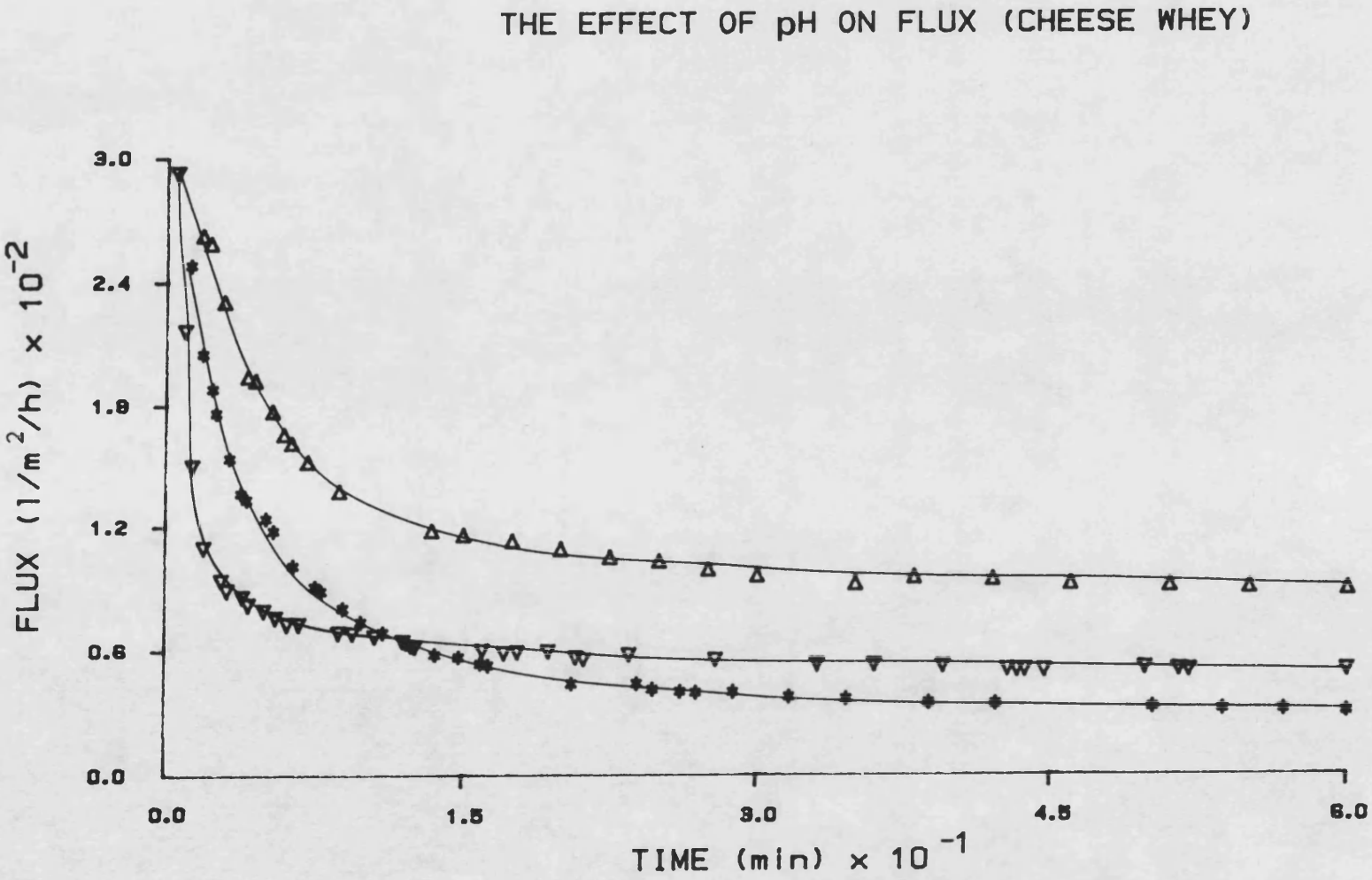


Fig.31: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein; C_b=1.5 g/ℓ; Δ pH=3.5; * pH=5.2; ∇ pH=8.5;

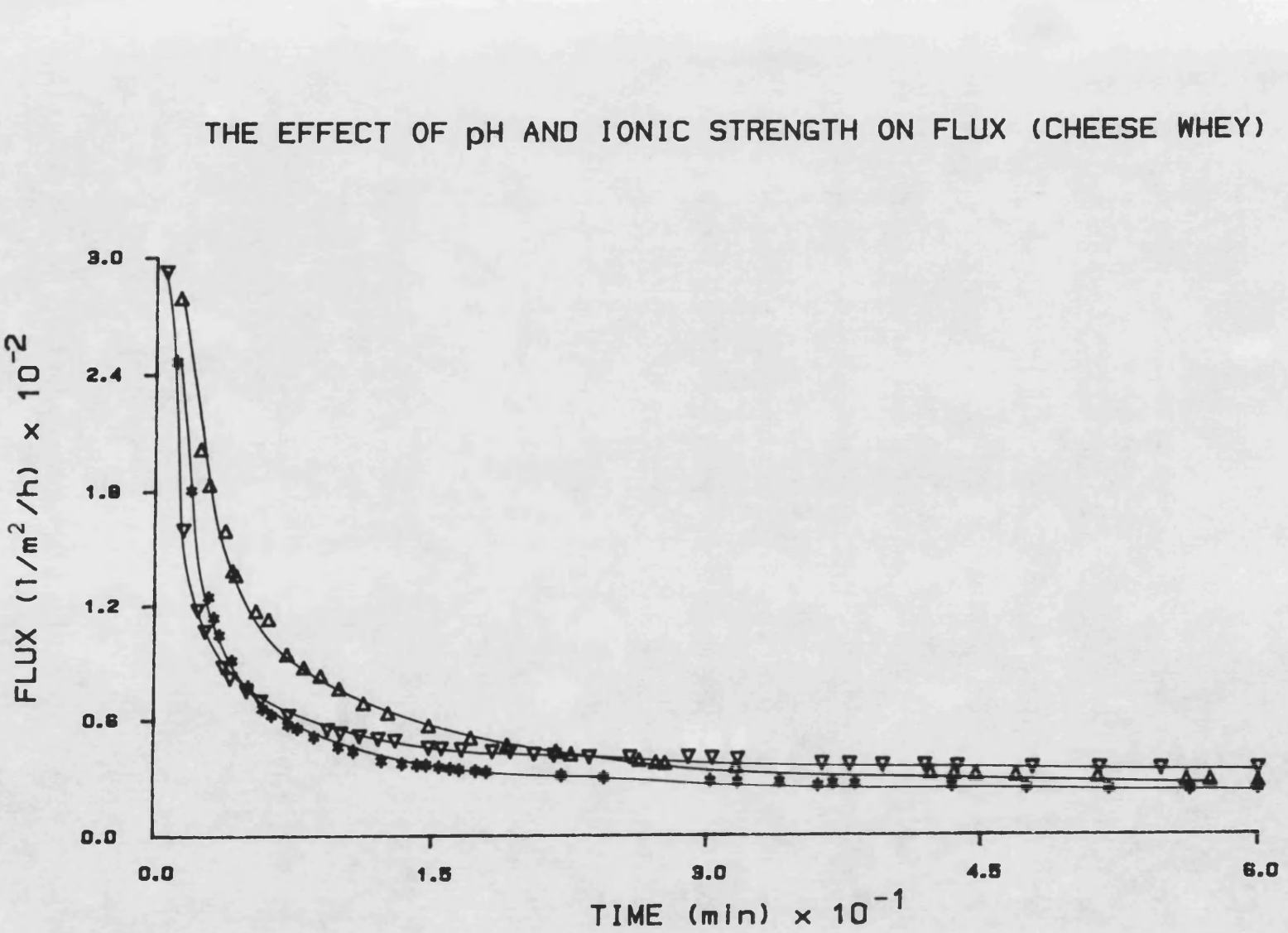


Fig.32: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein, C_h=1.5 g/ℓ; 0.1 M NaCℓ;
▽ pH=8.5; Δ pH=3.5; * pH=5.2;

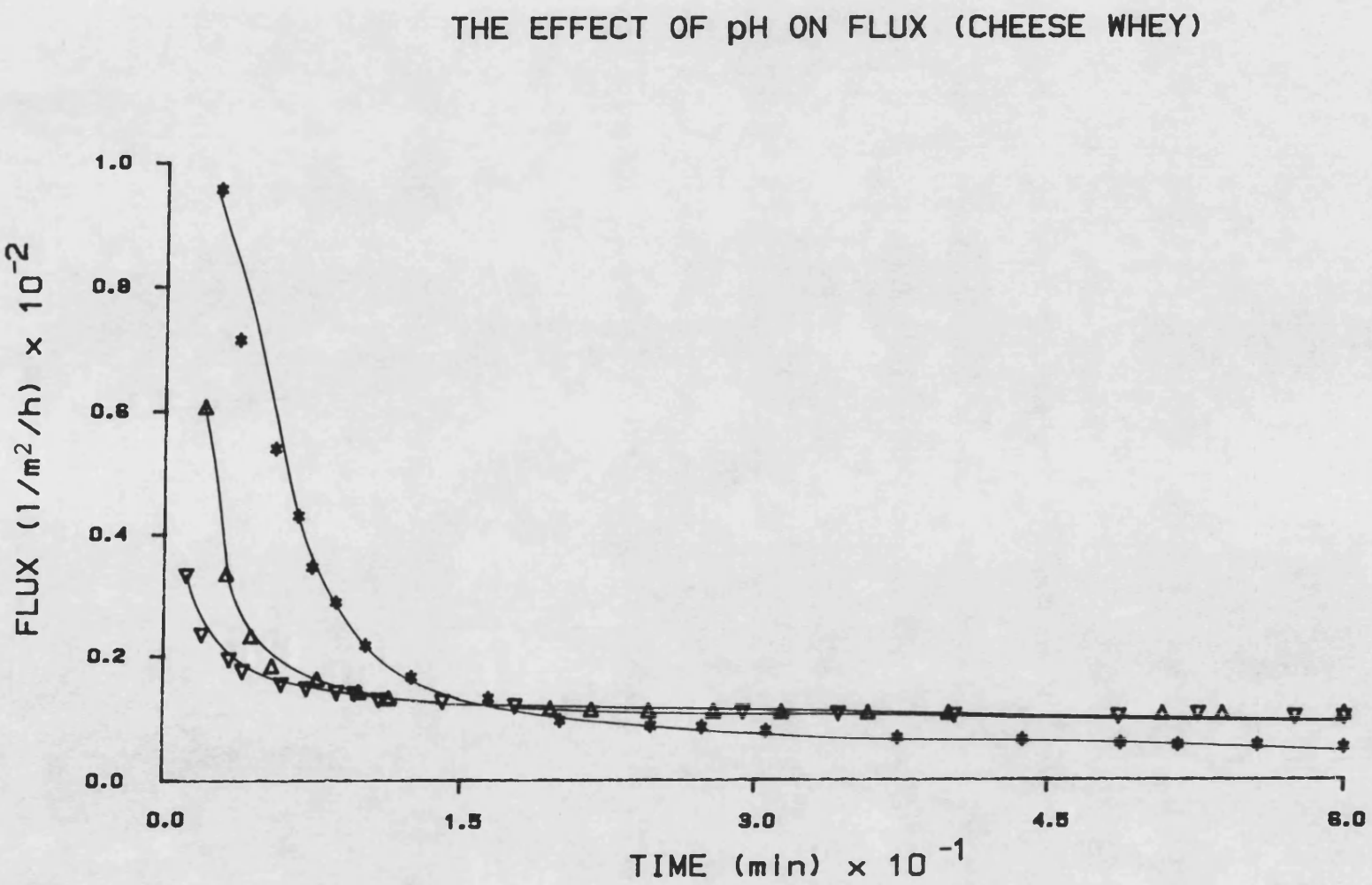


Fig.33: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein, C_b=13.4 g/ℓ; Δ pH=3.5; * pH=4.2; ▽ pH=8.5;

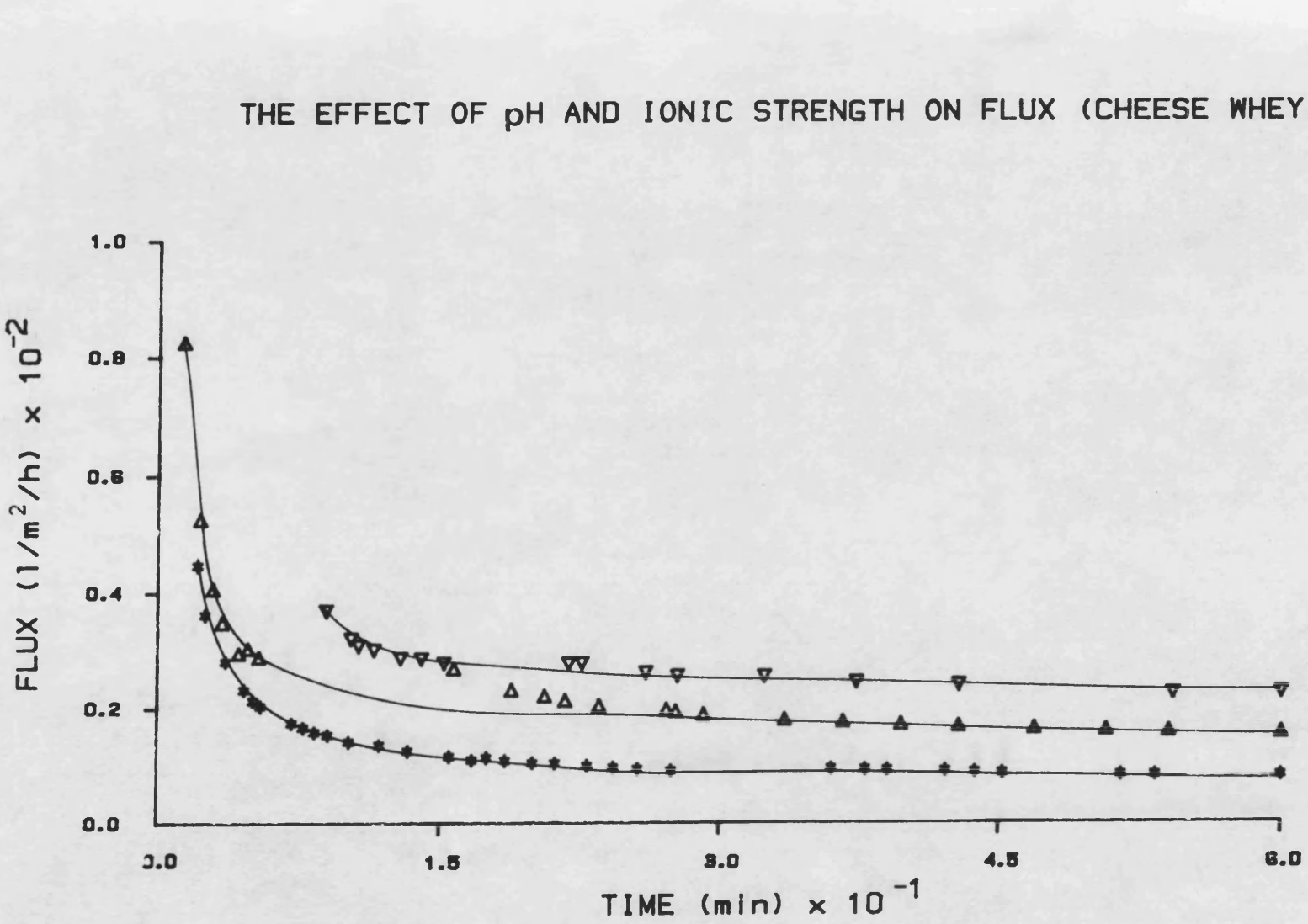


Fig.34: CFP-2D-3 Membrane; Recycle Flow Rate=0.4 ℓ/min; ΔP=20 kPa; T=10°C;
Feed: Whey Protein; C_b=13.0 g/ℓ; 0.1 M NaCℓ;
Δ pH-3.5; * pH-4.2; ▽ pH-8.5;

2.2 BiPro™

This set of experiments was performed with a thin channel flow cell mounted with a Nypor™ membrane. Nypor™ membranes are made of polyamide.

Bipro™ is a whey protein powder which has in principle the same composition as whey eluate and concentrate but differs in the way it has been processed. Whey eluate and concentrate are ultrafiltered cheese whey and the protein remains in its original solution whereas a Bipro™ solution it is redissolved, spray dried powder. However, one would expect them to show similar characteristics in protein transmission and flux.

Figs.35, 36 and 37 confirm this assumption. As with whey concentrate Bipro™ yielded a minimum flux around pH 4.4 which increased at a pH away from the I.E.P. Addition of salt resulted in the previously observed flux reduction. Notably, in spite of the presence of salt the flux still reflected changes in pH. In contradiction to the observations made with whey concentrate protein ,transmission of Bipro™ (Fig.38,39 and 40) showed a distinct minimum around the I.E.P in the presence and absence of salt.

Protein transmission with around 60% at a pH distinctly different from the I.E.P were in good agreement with the results of whey concentrate. The same applies to the case when salt was present.

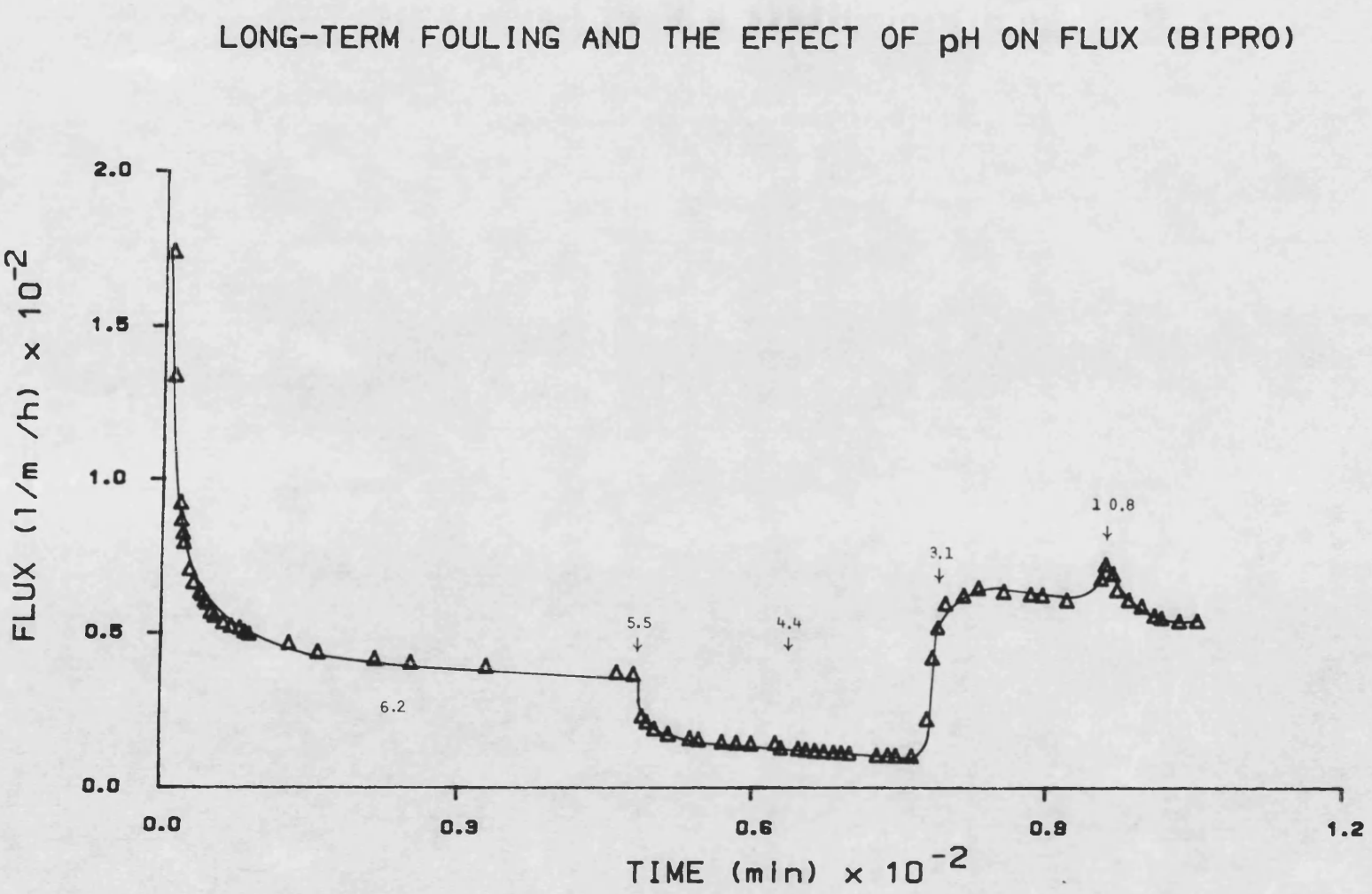


Fig.35: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Bipro™; C_b=10.2 g/ℓ; pH as shown;

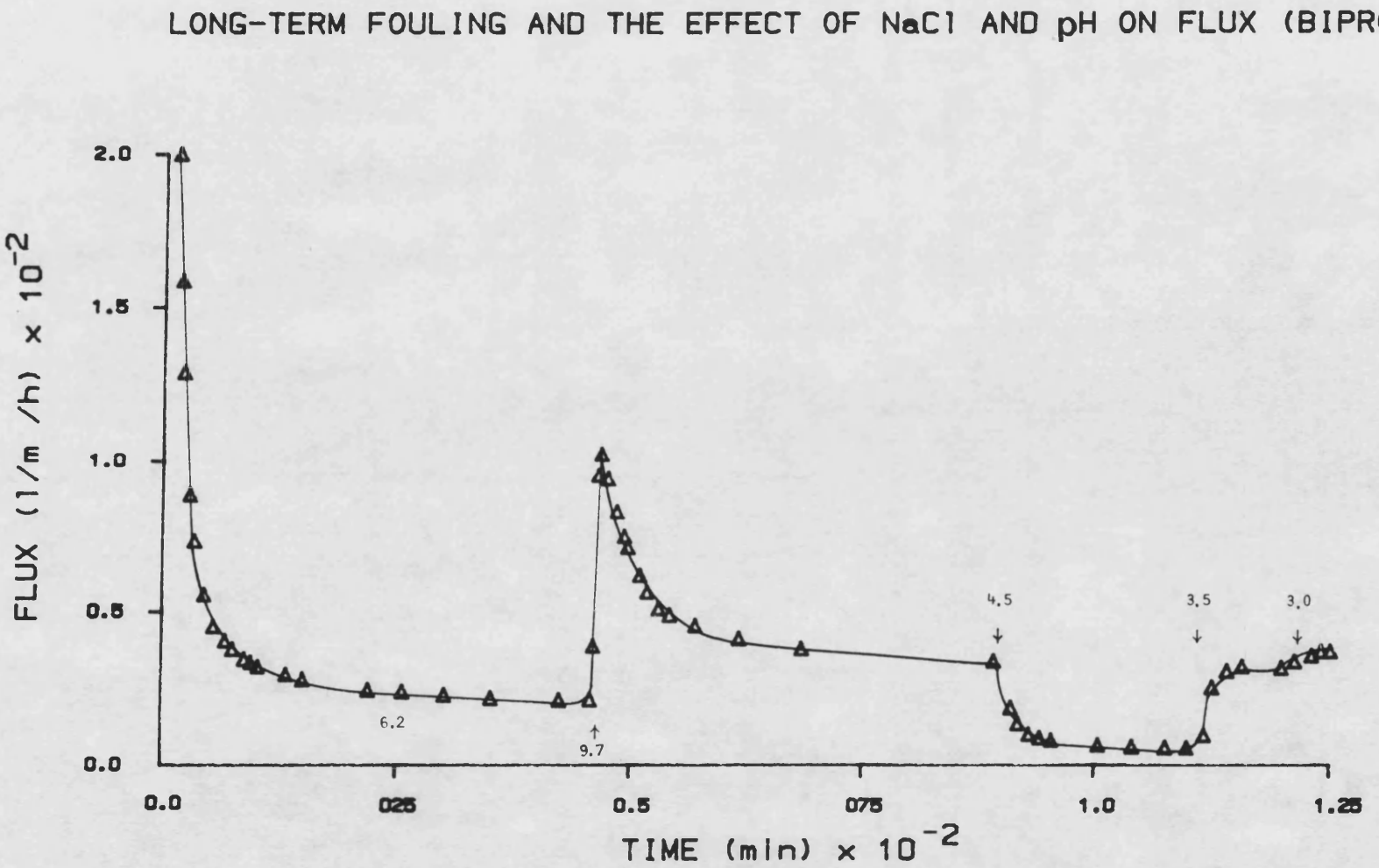


Fig.36: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Bipro™; C_b=10.2g/l, 0.05 M NaCl, pH as shown;

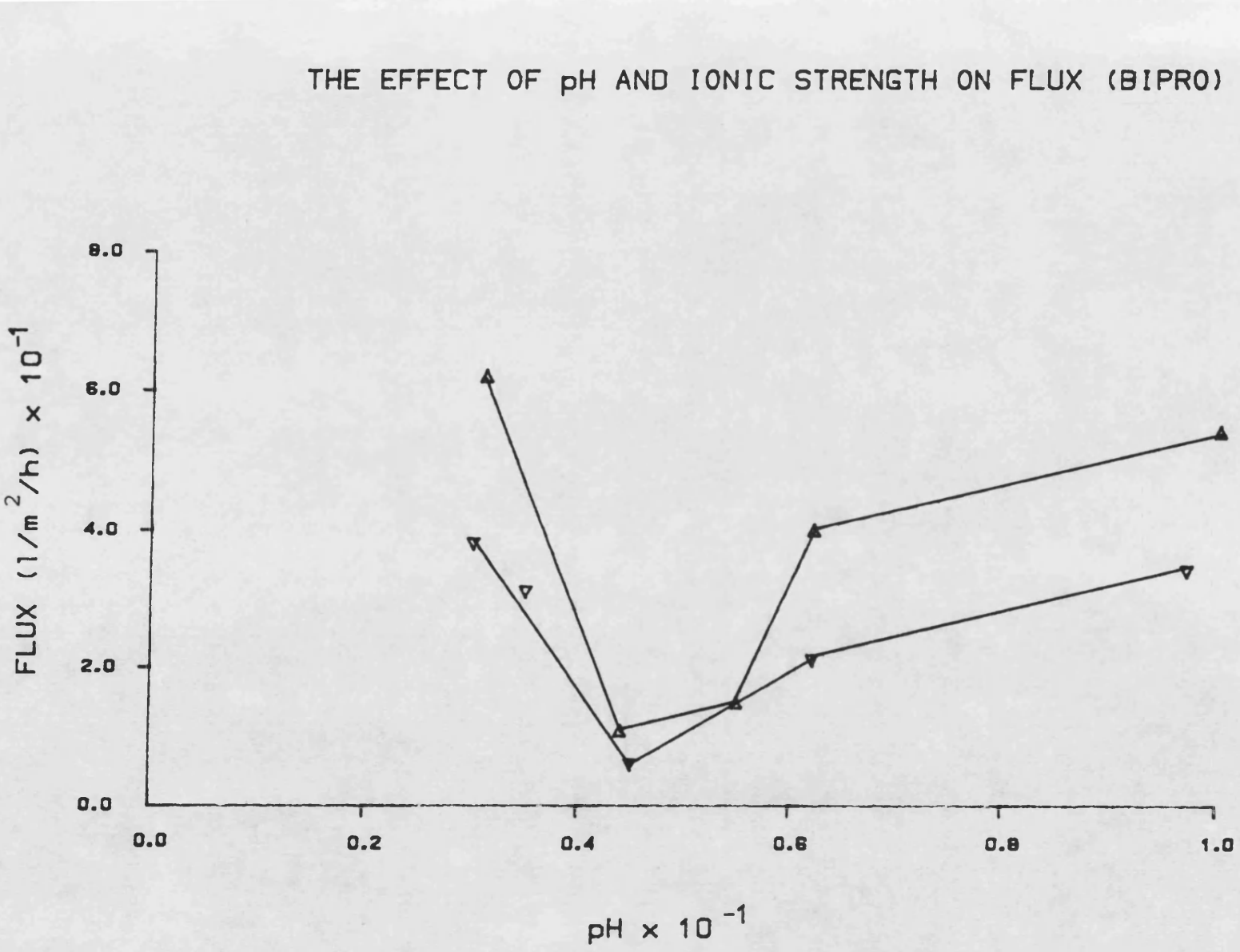


Fig.37: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa;
Feed: Bipro™; C_b=10.2g/l; Δ no salt; ▽ 0.05 M NaCl;

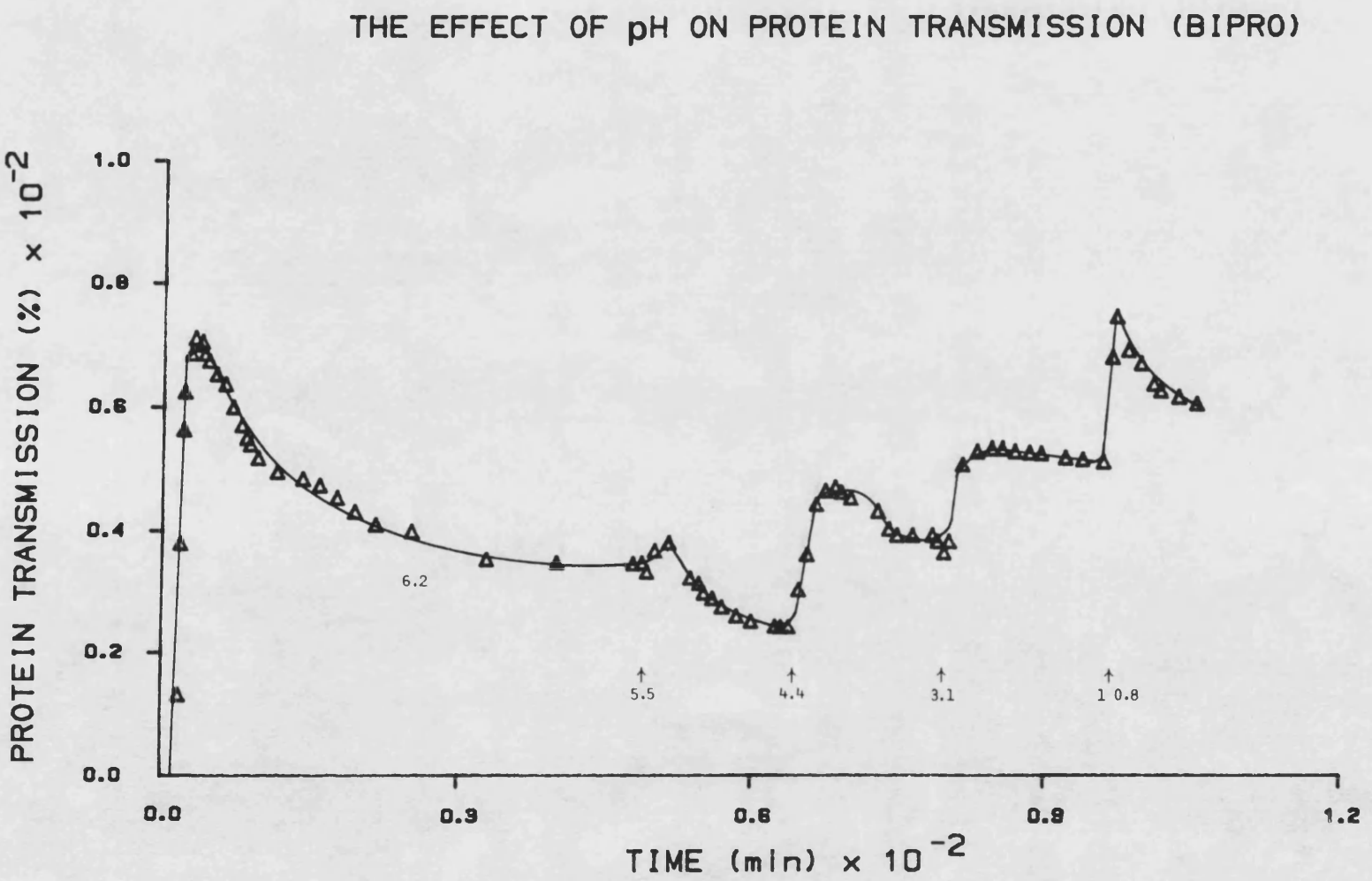


Fig.38: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Bipro™; C_b=10.2g/l; pH as shown;

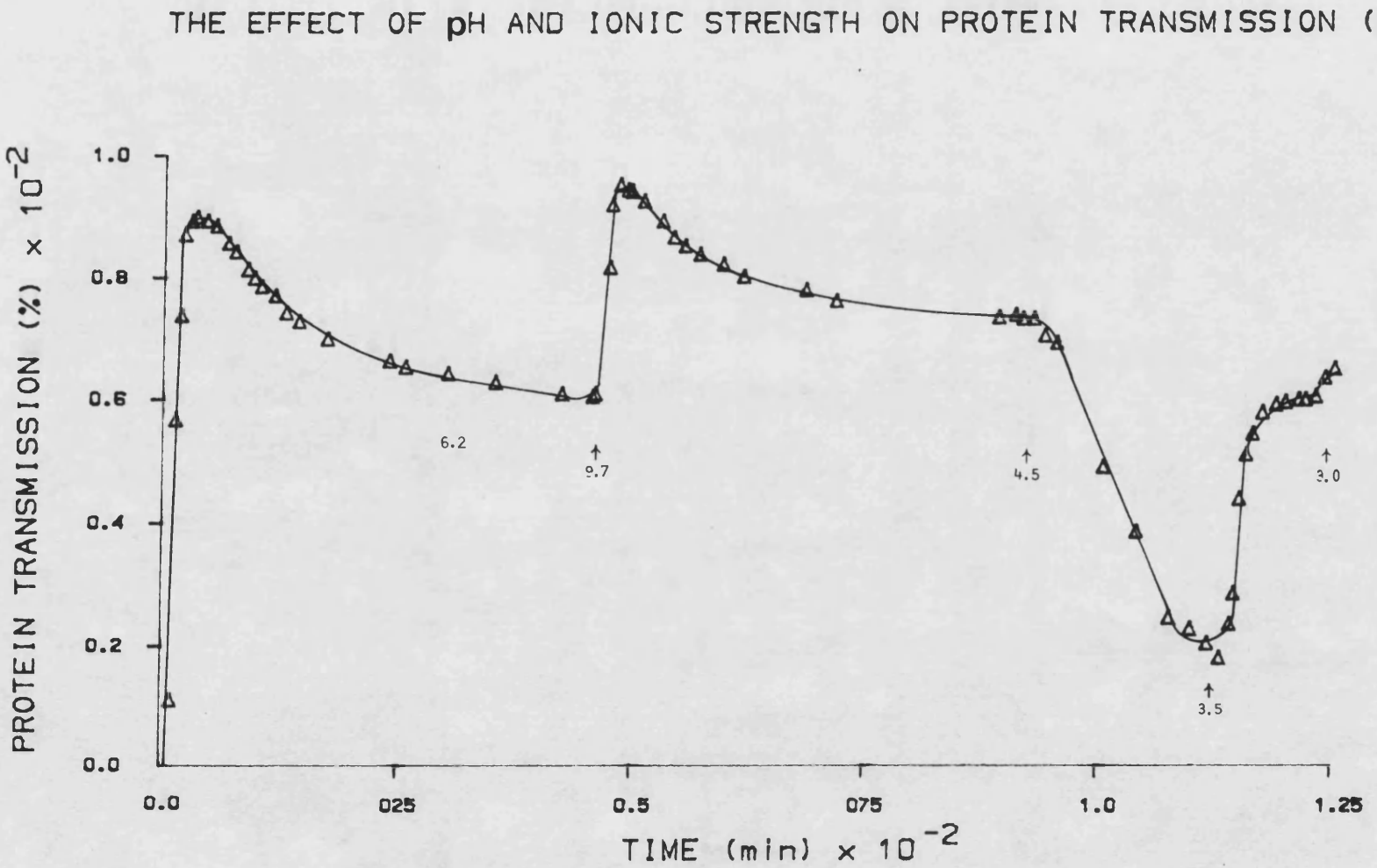


Fig.39: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa; T=10°C; Feed: Bipro™; C_b=10.2g/l; 0.05 M NaCl; pH as shown

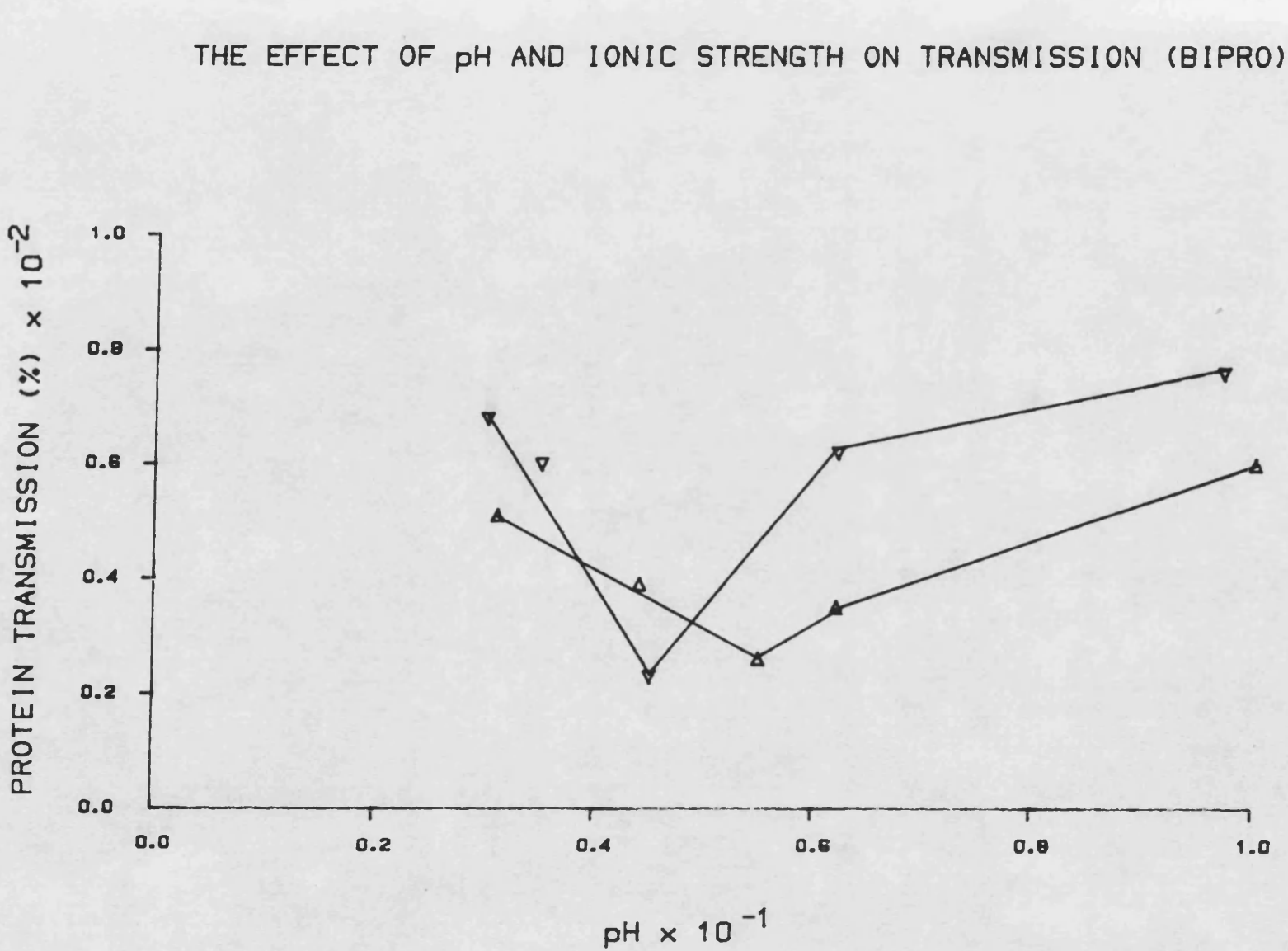


Fig.40: Nypor™ Membrane (0.2 μm); Recycle Flow Rate=0.5 ℓ/min; ΔP=20 kPa; Feed: Bipro™; C_b=10.2; Δ no salt; ∇ 0.05 M NaCl,

TABLE 12 Summary of the Results Presented in Sections 1 and 2

Protein	I.E.P	Molecular Weight	Concentration g/ℓ	Buffer mol/ℓ	pH	Flux ℓ/m²/h	Transmission %	Remarks
BSA	4.8	69,000	7.5	-	2.8	80 ↑	35 ↓	Flux is lowest at I.E.P
				-	4.7	30	70	Transmission is highest
				-	9.0	105 ↓	55 ↑	at I.E.P
Casein	4.6	121,700	3.3	-	2.9	45 ↓	30 ↑	Flux does not vary signific-
				-	5.6	50 ↓	25	antly with pH. Transmission
				-	6.5	60 ↓	36 ↓	exhibits a moderate minimum
				-	7.8	60 ↓	40 ↓	around the I.E.P. Dissolved
				-	9.1	65 ↓	38 ↓	Casein is a colloidal suspension
Pepsin	<2	34,700	5.0	-	2.5	120 ↑	23	Pepsin is negatively charged
				-	3.1	380 ↓	32	over the whole range of pH.
				-	3.4	350 ↓	32	At pH>10 denaturation occurs.
				-	5.4	260	32	Flux is determined by est-
				-	7.7	290 ↓	32	ablished fouling layer of
				-	10.0	300 ↓	37	whey proteins and reflects
				-	11.0	260 ↓	40	their characteristics compared with cheese whey.

Ovalbumin	4.6	46,000	4.0	-	2.9	51 ↑	22 ↓	Flux is lowest at I.E.P.
				-	3.7	51 ↓	54 ↓	Transmission is highest
				-	4.9	23	78	at I.E.P
				-	6.7	44 ↓	26 ↑	
				-	9.2	65 ↓	40 ↓	
Ovalbumin	4.6	46,000	4.0	Phosphate	2.0	25 ↓	48 ↓	Flux does not vary
				0.1	5.4	25 ↓	62 ↓	significantly with pH.
				0.1	7.7	30 ↓	66 ↓	Transmission does not show
				0.1	8.8	33 ↓	70 ↓	a minimum or maximum
				0.1	9.2	38 ↓	72 ↓	
Ovalbumin	4.6	46,000	4.0	NaCℓ	2.2	27 ↑	36 ↓	Flux is lowest at I.E.P.
				0.1	4.8	12	37 ↓	Transmission does not show
				0.1	5.9	19 ↓	45 ↓	a minimum or a maximum
				0.1	9.2	32 ↓	62 ↓	
Ovalbumin	4.6	46,000	4.0	-	9.2	65 ↑	40 ↓	Flux is highest in absence of
				Phosphate	9.2	38 ↓	70	salt. Transmission is highest
				NaCℓ	9.2	32 ↓	62 ↑	in presence of phosphate ions
Whey		14,000		-	3.5	95 ↑	55 ↓	Flux is lowest at I.E.P
Concentrate	4.2-5.2	35,000	1.5	-	5.2	35	80	Transmission is highest at
and Eluate		69,000		-	8.5	60 ↓	48 ↑	I.E.P.

Whey Concentrate and Eluate	4.2-5.2	14,000 35,000 69,000	1.5	NaCℓ 0.1 0.1	3.5 5.2 8.5	30 ↓ 32 28 ↑	64 ↑ 56 60 ↓	Flux does not vary significantly with pH. Transmission is lowest at I.E.P.
Whey Concentrate and Eluate	4.2-5.2	14,000 35,000 69,000	14	- - -	3.5 4.2 8.5	12 ↑ 8 12 ↓	60 ↓ 65 55 ↑	Flux is lowest at I.E.P. Transmission is highest at I.E.P.
Whey Concentrate and Eluate	4.2-5.2	14,000 35,000 69,000	14	NaCℓ 0.1 0.1	3.5 5.2 8.5	18 ↑ 10 25 ↓	62 ↑ 60 ↓ 58 ↓	Flux is lowest at I.E.P. Transmission does not show a minimum or maximum
BiPro™	4.2-5.2	14,000 35,000 69,000	10.2	- - - - -	3.1 4.4 5.5 6.2 10.8	62 ↑ 11 15 ↓ 40 ↓ 54 ↓	51 ↑ 39 ↓ 26 35 ↓ 60 ↓	Flux is lowest at I.E.P. Transmission is lowest at I.E.P.
BiPro™	4.2-5.2	14,000 35,000 69,000	10.2	NaCℓ 0.05 0.05 0.05 0.05	3.0 3.5 4.5 6.2 9.7	38 ↑ 31 ↓ 6 21 ↓ 34 ↓	68 ↑ 60 ↓ 23 62 ↓ 76 ↓	Flux is lowest at I.E.P. Transmission is lowest at I.E.P.

All experiments were performed with the hollow fibre cartridge except BiPro™ which was performed with the thin channel flow cell fitted with a Nypor™ membrane.

3. DISCUSSION

Table 12 summarises the results and observations presented in sections 1 and 2. All values are steady state values. The table also contains that information on the proteins used which is important for the following discussion.

Amongst other factors such as hydrodynamic conditions, hydrophobicity of the membrane and temperature, which are not a subject of this discussion, protein adsorption apparently has a major impact on membrane performance. Both flux and transmission decline with time due to some mechanisms which have not been classified yet but are generally referred to as fouling and consolidation of the fouling layer.

The results of this work show clearly that membrane permeability and protein transmission are strongly affected by pH and ionic strength.

This has been reported already in the literature but the information available seemed to be inconsistent. Fane et al (1983), Suki et al (1984) and Aimar et al (1986) found a maximum hydraulic resistance around the I.E.P, whilst Matthiasson (1984) reported an increasing membrane permeability with decreasing pH and Hoare et al (1986) measured a maximum flux around the I.E.P.

More consistent are the results published on protein transmission or rejection respectively, although only little work has been published for microporous membranes. Fane et al (1983) showed that rejection in the case of a partially permeable membrane is lowest around the I.E.P and Le and Atkinson (1985) improved enzyme transmission by adding phosphate salt.

Also results published on adsorption profiles with respect to the ionic environment are not uniform. Fane et al (1983) and Zeman (1983) observed an increasing adsorption with increasing ionic strength, whereas Matthiasson (1983) found maximum adsorption in the absence of salt.

These observations only appear to be contrary but to the author's belief cannot be compared with each other. The experiments mentioned above were performed with membranes made of different materials and had different surface properties. Suki et al (1984) showed that a discussion on the influence of pH on protein adsorption cannot

neglect the type of membrane used. They pointed out that surface heterogeneity in terms of porosity and mean pore size have a major impact on protein adsorption to membranes.

Le et al (1984a) also demonstrated that the ratio of pore size to particle size strongly affects the transmembrane permeability.

Summarising the results and observations published in the literature and those presented in this work one concludes that an interpretation of the fouling phenomenon has to take into account the following points:

- nature of the protein, mainly particle size and the I.E.P.
- distinction between a solution and suspension
- pH
- ionic strength and type of buffer used
- membrane material
- properties of the membrane surface, namely porosity and pore size.

Furthermore, it is common to attribute flux and protein transmission decline to the same mechanism. To the author's belief flux and transmission are not necessarily determined by the same process and it is suggested that both cases should be considered separately.

Flux is determined by the increasing hydraulic resistance produced by the fouling layer and the growing consolidation of the layer. Hence, the main factor is the porosity of the fouling layer which, in turn, depends on the conformation of the particles from which it is formed.

The layer will be closely packed when proteins have a compact conformation as is the case at the I.E.P. Transmembrane permeability should be at a minimum. At a pH below or above the I.E.P proteins are enlarged and repulsion might occur because of the same net charge. The layer will be more porous thus flux will be increased. In the presence of salt net charges will be screened to some extent and the layer becomes denser

again. Flux should be reduced. Adding salt to a protein solution adjusted to the I.E.P could be ineffective for the flux or make the layer even more densely packed and reducing thereby the permeability further.

Flux is also determined by the ratio of pore size to particle size. If the pore size is smaller than the diameter of the particle only deposition on the membrane surface should occur provided proteins maintain their conformation when convected to the surface and do not break up into sub-units. If the pore size is larger than the particle size pore blocking becomes likely and the freely permeable area is diminished thus flux is reduced. Protein transmission, on the other hand, is determined by protein adsorption to the membrane and the pore size. As discussed in part I, polymeric surfaces immersed in an aqueous phase are electrically charged. The primary charge is balanced by counter ions producing a diffuse double layer which could affect protein transmission in two ways. Firstly, the diffuse layer extends into the aqueous phase and is likely to reduce the actual pore size. Thus, less proteins are transmitted.

Secondly, the diffuse double layer is charged and so are the proteins. This suggests an interaction of the double layer and the proteins which, in turn, is likely to affect protein transmission. The nature of the interaction will depend on parameters which influence the charge conditions, namely pH and ionic strength. It is necessary to distinguish between three possible situations:

1. Protein and diffuse double layer carry the same charge.

   Presumably proteins are repelled by the double layer to some extent. Protein transmission can be expected to be low.

2. Protein and diffuse double layer are oppositely charged.

   An opposite charge suggests a strong attraction and it is very likely that the proteins interact strongly with the diffuse layer. The question remains whether the proteins maintain their globular conformation or if they open up their tertiary structure into a fibrous conformation and spread in some kind of mesh across the membrane surface. Presumably the fibrous ropes also spread over the pores thus reducing the actual pore size and allowing less proteins to transmit. Recent TEM photographs support this hypothesis.

However, once the first layer of protein is established it carries the same charge as the proteins in solution and repulsion should retard a further build-up of layers and transmission should remain constant.

3. Proteins are at the I.E.P

At the I.E.P proteins do not have a net charge and little or no interaction with the diffuse layer should occur. Transmission should be highest compared to cases where the pH is away from the I.E.P. On the other hand, particle deposition on the membrane surface is still evident, preventing a 100% transmission.

4. Cases where salt is present.

When the bulk solution contains a high ionic concentration (high ionic strength), the diffuse double layer is compacted so that it occupies a smaller volume and does not extend as far into the solution; hence, the permeable pore area is enlarged. Transmission should be generally higher in the presence of salt. Simultaneously, the charges of the proteins are screened by the salt and less interaction with the diffuse layer is likely to occur. This also favours a higher transmission and, furthermore, changing the pH should not result in extreme transmission differences.

The graphs presented in the former two sections and the steady state values listed in Table 12 show that BSA, ovalbumin, whey concentrate and BiPro^{TM} yielded in general, a higher flux at a pH away from the I.E.P which is in accordance with the assumptions made before. Casein seems to be an exception. Flux does not vary significantly with pH. Unlike the other proteins, casein is only sparingly soluble in water and produces a colloidal suspension rather than a solution. Apart from the fact that colloids are bigger than dissolved proteins and the uniform flux could be a result of the pore size/particle size ratio, it also could be a consequence of weaker charge effects. Although colloidal particles can develop charges, which are counter-balanced by a diffuse double layer, they do not have net charges and are therefore less affected by a change in pH. Thus, the porosity of the flux restricting layer is likely to remain constant over the range of pH.

Pepsin also shows a minimum flux around the I.E.P but at pH 5.4 a second, more moderate minimum was observed. This is somewhat surprising since pepsin is negatively

charged over the whole range of pH and should not produce a flux variation. Presumably the flux profile reflects more the properties of the established fouling layer of previous experiments rather than the properties of the pepsin solution. A similar observation was made in part I when the water flux profile was changed due to altered membrane characteristics. Prior to pepsin the membrane was exposed to whey proteins which have an I.E.P around pH5. This could be an explanation for the second flux decline.

As predicted, adding salt resulted generally in a flux depression except when whey proteins were at a high concentration. In this case supplementary salt almost doubled the flux. Probably at this high protein concentration salt induces some salting out. The precipitated particles might be large enough to bridge the pores rather than plugging them. It is also possible that the enlarged particle size forms a more porous layer exhibiting a lower resistance to the liquid flow. Both processes would yield in a higher flux.

Worth mentioning is the unusually high flux observed with the pepsin solution which might be a result of the special membrane configuration used in this experiment. The membrane was fitted so that the more open side faced the feed. Therefore the high flux could be the result of more favourable pore size/particle size ratio. Le et al (1984) also noted an enhanced flux when the membrane was used in the reverse configuration. Coupled with this the highly negatively charged pepsin molecules might have caused repulsion amongst each other, hence the deposit layer was less dense.

The results of protein transmission are also in good agreement with the predictions deduced from the double layer theory. BSA, ovalbumin and whey concentrate showed a maximum transmission around the I.E.P. At a pH different from the I.E.P more proteins were rejected. Most surprisingly, in contrast to whey concentrate, BiPro^{TM} transmitted at the lowest rate around the I.E.P, although both are of the same composition. The conjecture that the different membrane systems used might give an explanation can be largely excluded since all other observations conform. However, both products have been processed in a different way which is more likely to be the explanation.

BiPro^{TM} was spray-dried which suggests that the protein conformation might have been altered in some way. This apparently does not affect the filter properties in general but in the case when the solution was adjusted to the I.E.P. It is possible that agglomerates are formed being larger in size than the pores.

The transmission of pepsin was constant at pH 3-8. This again is in good agreement with the double-layer theory. Pepsin has an I.E.P < 2 and is negatively charged over the whole range of pH, hence transmission should be constant. The observed low transmission with 30% is a further manifestation of the double-layer theory since it predicts a low transmission for cases away from the I.E.P.

The enhanced transmission at pH > 10 is presumably due to denaturation of pepsin. At pH 2.5 the protein develops its proteolytic properties and interacts with the established fouling layer of previous experiments . Protein transmission is reduced.

The transmission of casein showed a minimum around the I.E.P which might be due to its poor solubility. It is possible that under this environmental condition agglomerates are formed which are partially larger than the pores.

Adding salt to the proteinaceous solutions increased the protein transmission in all cases but at the I.E.P which is once more in accordance with the double-layer theory. It is notable that phosphate salt has a more pronounced effect than NaCℓ. Apparently the doubly charged phosphate ion compacts the diffuse layer more than NaCℓ does. Another plausible explanation is that phosphate has ion-exchange properties and can displace loosely attached foulants, hence inhibiting protein binding to some extent (Le, 1982).

PART III

Membrane Fouling at a Low Transmembrane Pressure

Whereas the effect of shear rate at the membrane surface has been investigated intensively and led to the generally accepted theory that increasing the shear results in a higher flux, the effect of transmembrane pressure on protein transmission and rejection respectively attracted less attention. Besides, reported results in the literature are inconsistent.

Goldsmith (1971) found a general decrease in rejection with increasing transmembrane pressure for very dilute solutions of Dextran. For more concentrated solutions he observed an enhanced rejection with increasing pressure. He proposed a compaction of the membrane skin with increasing pressure which results in a reduced pore size. An increased rejection, so he claimed, is more pronounced for relatively highly concentrated solutions in which case a rise in operating pressure increases the solute concentration at the membrane surface only slightly.

Spiegler and Kedem (1966) noted that under conditions where diffusive flow is predominant the observed retention tended to be zero. They therefore assumed that at very low pressure diffusion rather than convection is the predominant mechanism of solute transport and leads to an enhanced solute transmission. Papamichael and Kula (1987) measured the retention of polyethylene glycol with increasing operating pressure. They observed zero retention when a convective flux was absent and supported Spiegler and Kedem's theory whilst dismissing the concept of membrane compaction. All agreed with the observation that enhanced solute transmission occurs when transmembrane pressure was lowest.

Since the observations mentioned above were made with ultrafiltration membranes it was interesting to see whether a low operating pressure could help to improve protein transmission through a microporous membrane.

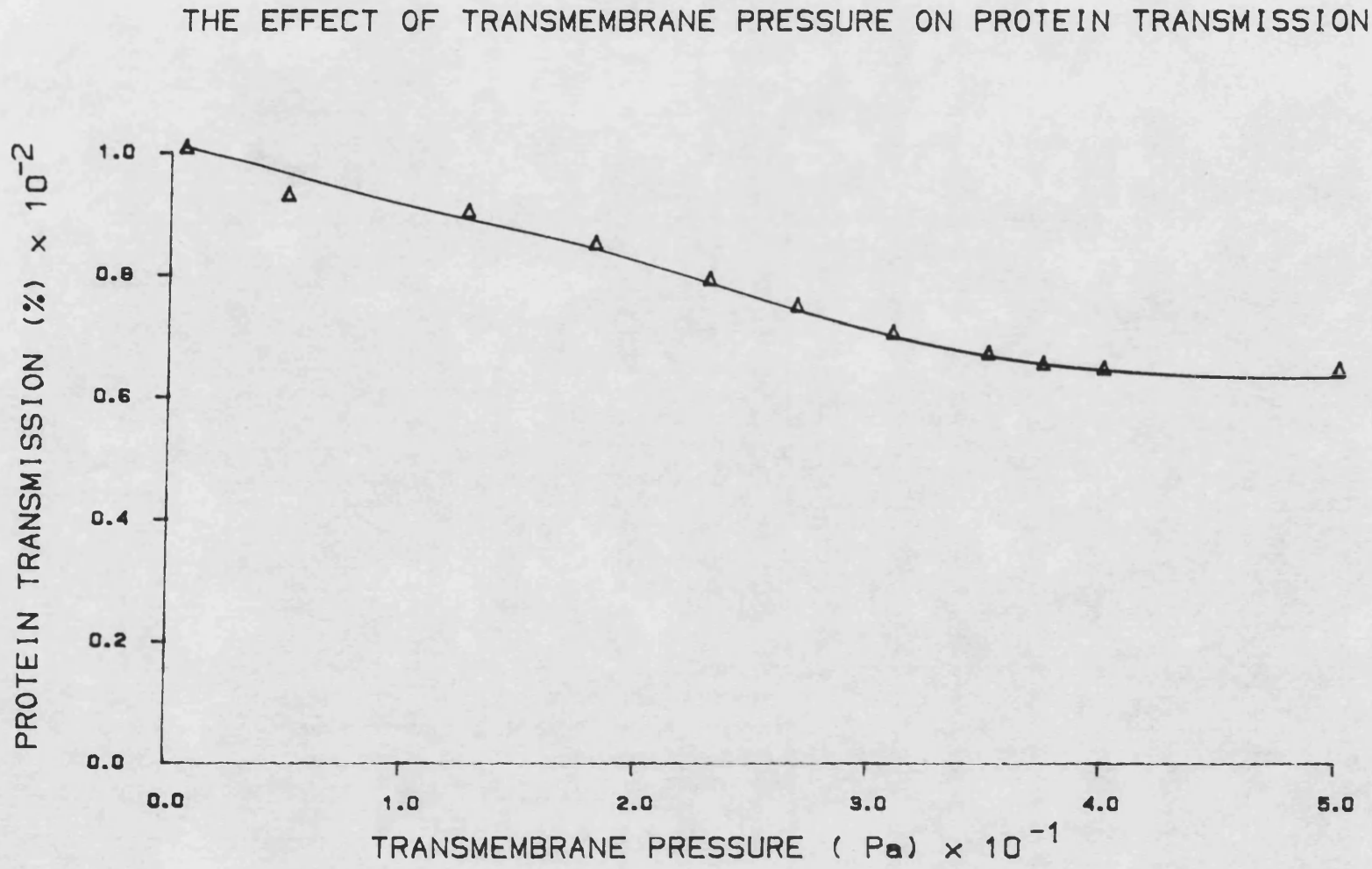


Fig.41: CFP-2E-3 Membrane; Flux=50ℓ/m²/h; T=10°C;
Feed: Whey Protein; C_b=2.8g/ℓ; 0.1 M Potassium Phosphate; pH=8.5;

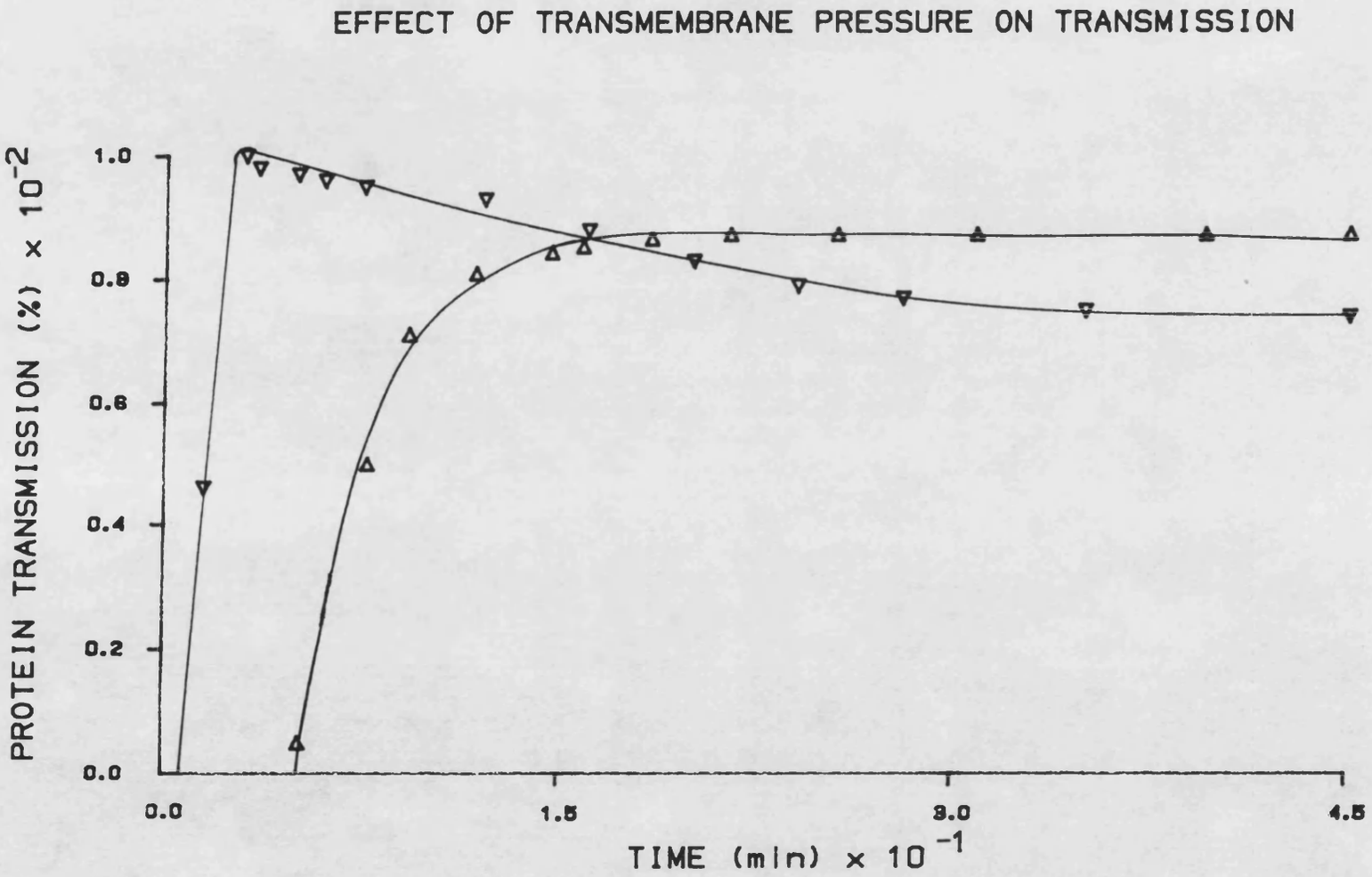


Fig.42: CFP-2E-3 Membrane; Recycle Flow Rate=0.5ℓ/min; T=10°C;
Feed: Whey Protein; 0.1 M Potassium Phosphate; pH=8.5;
Δ C_b=7.8 g/ℓ, ΔP=1130 Pa; ∇ C_b=6.6 g/ℓ, ΔP=20 kPa;

1. The Effect of Transmembrane Pressure on Protein Transmission

Fig.41 demonstrates clearly that protein transmission is a function of transmembrane pressure. Protein transmission was 100% at a very low operating pressure and decreased over a range of increasing pressure. In the higher pressure region transmission became independent of the pressure and remained constant. This result is in good agreement with Papamichael and Kula's observations who also found a pressure independent rejection at a pressure around 40 kPa.

Fig.42 compares the transmission profiles of two entire filtration runs at similar environmental conditions but different pressures. At a pressure of 20 kPa maximum transmission occurred shortly after the run was started and decreased thereafter to a steady state value. Protein transmission at a pressure of 1130 Pa showed a lag-phase before inclining very slowly to a maximum of 87%. No following decline was observed.

2. The Effect of Solution Properties on Protein Transmission and Flux at a Low Transmembrane Pressure

Although the previous results showed clearly that a low pressure allows more solute to pass through the membrane the question remained as to what extent the solution properties themselves contributed to the observed higher protein transmission, and furthermore, would an alteration in environmental composition yield a maximum transmission of 100%?

Fig.43 illustrates that charge effects still exhibit a major impact on protein transmission. Whilst in the presence of phosphate salt no rejection was detectable, transmission was only 80% when no salt was added. Coupled with this at a low ionic concentration some loss of transmission was observed. Nevertheless, even in the absence of salt a low operating pressure induced a considerable improvement. A whey protein solution of a lower concentration adjusted to the same pH but filtered at a higher pressure showed poorer transmission and the steady state value was only 48%. (compare Fig.27).

On the other hand, a low transmembrane pressure and supplementary salt yields a very low flux as displayed in Fig.44. The final flux in the presence of phosphate was only 15 $\ell/m^2/h$ in the absence of salt. This is not too discouraging because the flux of a

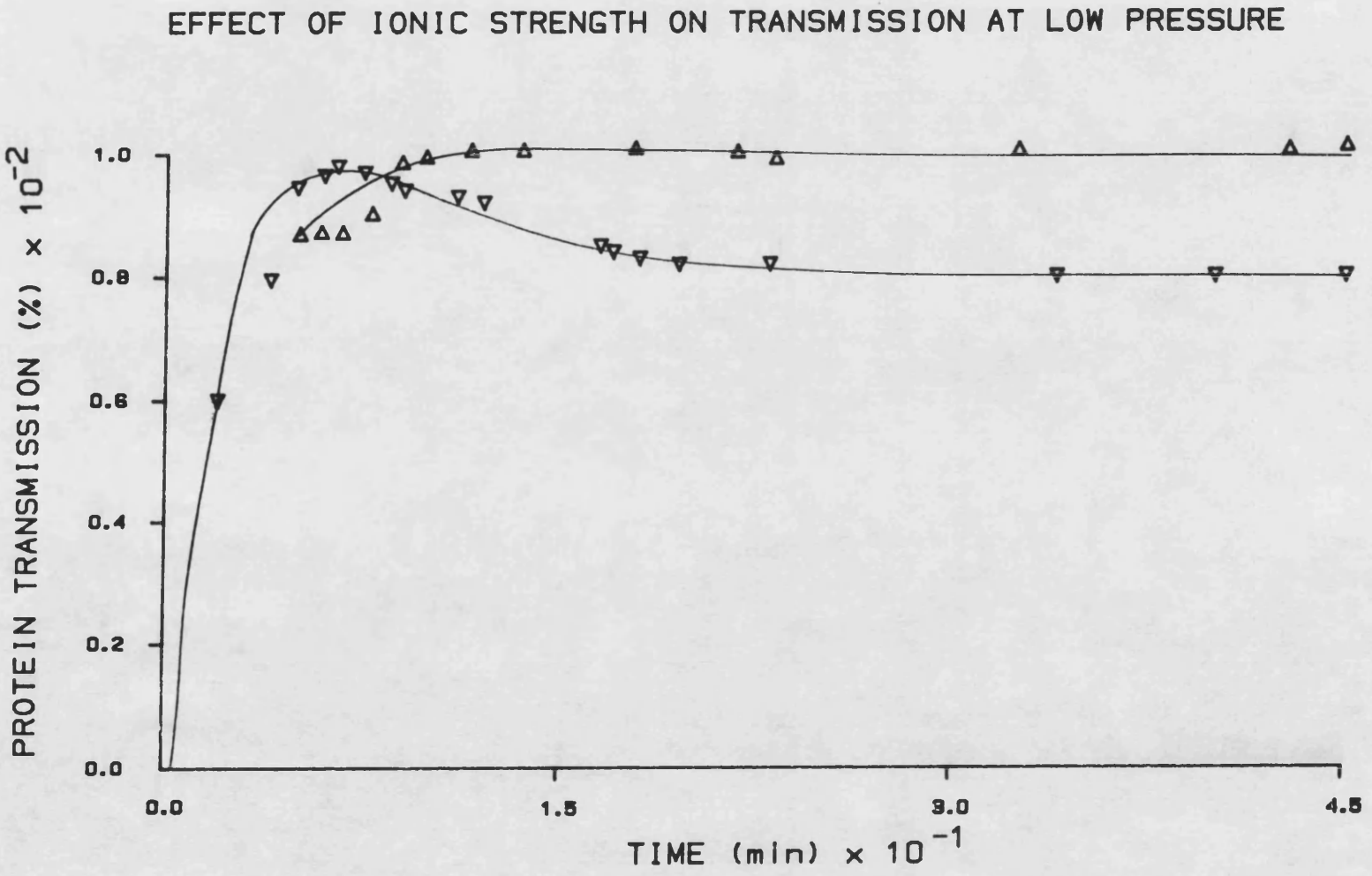


Fig.43: CFP-2E-3 Membrane; Recycle Flow Rate=0.5 ℓ/min; ΔP=1130Pa;T=10°C;
Feed: Whey Protein; C_b=5g/ℓ; pH=8.5;
▽ no salt; Δ 0.1 M Potassium Phosphate;

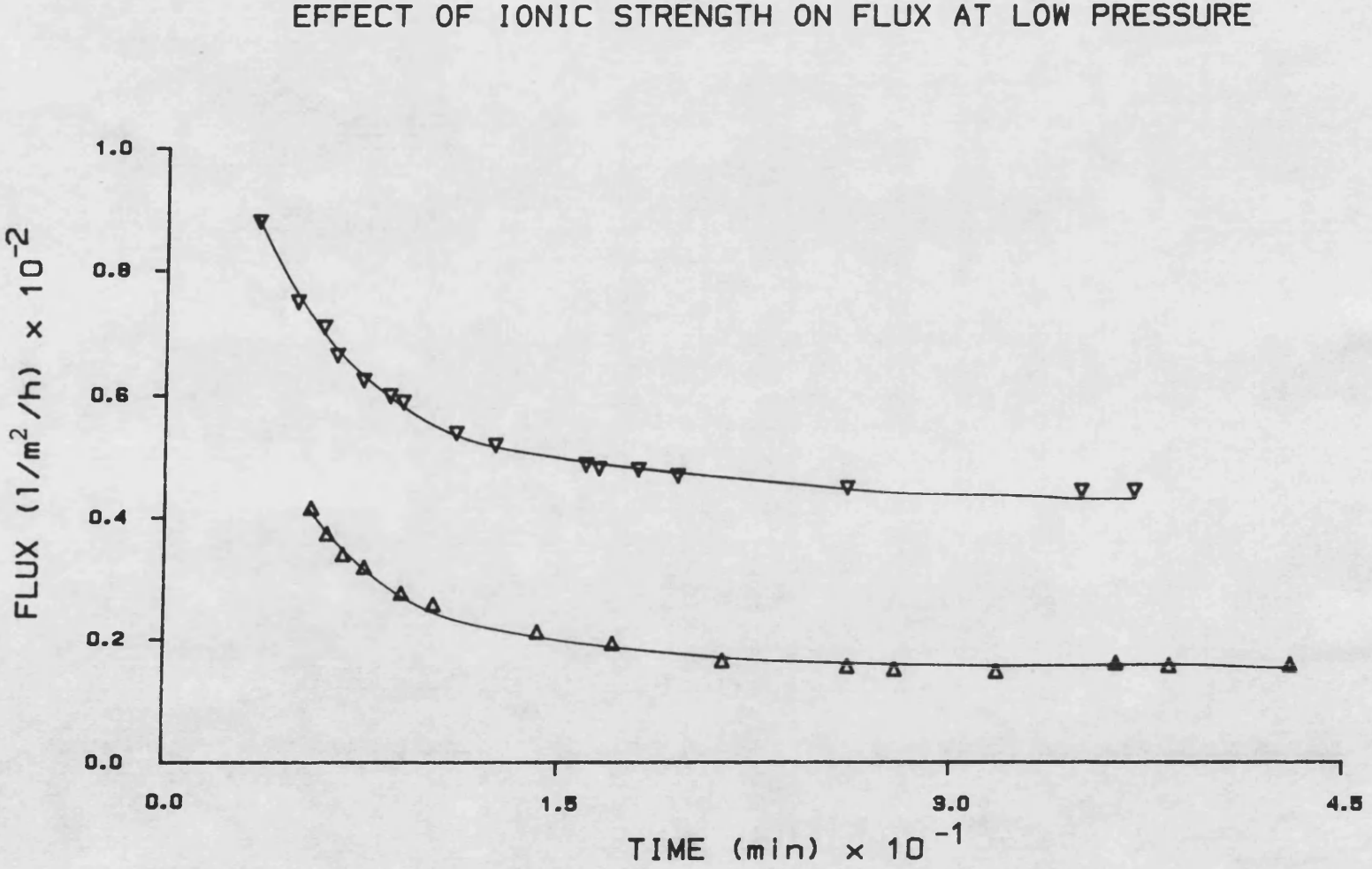


Fig.44: CFP-2E-3 Membrane; Recycling Flow Rate=0.5 ℓ/min; ΔP=1130Pa;T=10°C;
Feed: Whey Protein; C_b=5g/ℓ; pH 8.5;
▽ no salt; Δ 0.1 M Potassium Phosphate;

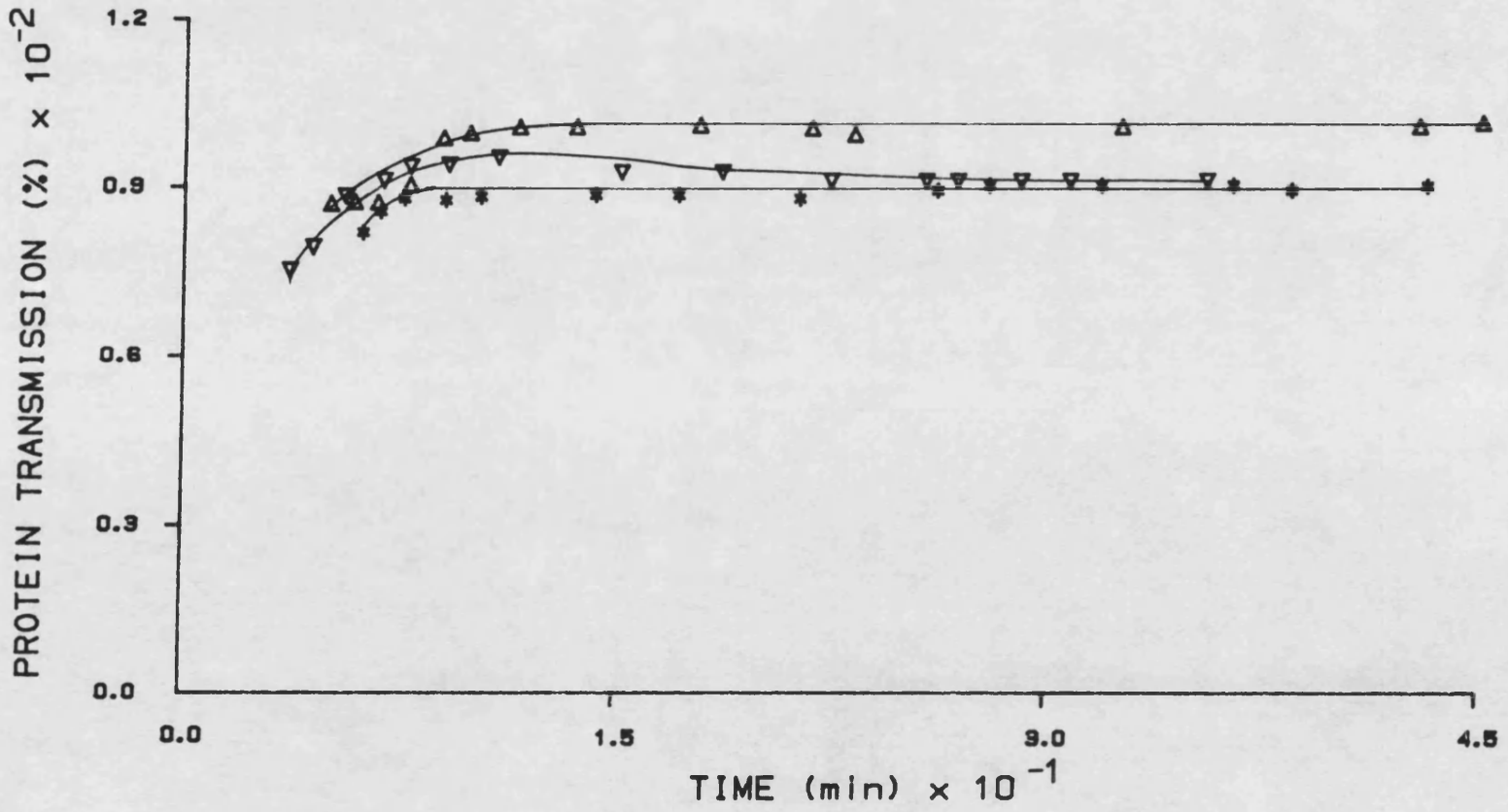


Fig.45: CFP-2E-3 Membrane; Recycling Flow Rate=0.5 ℓ/min; ΔP=1130Pa;T=10°C;
Feed: Whey Protein; 0.1 M Potassium Phosphate; pH=8.5;
* 5 g/ℓ; Δ 9.5 g/ℓ; ▽ 14.5 g/ℓ;

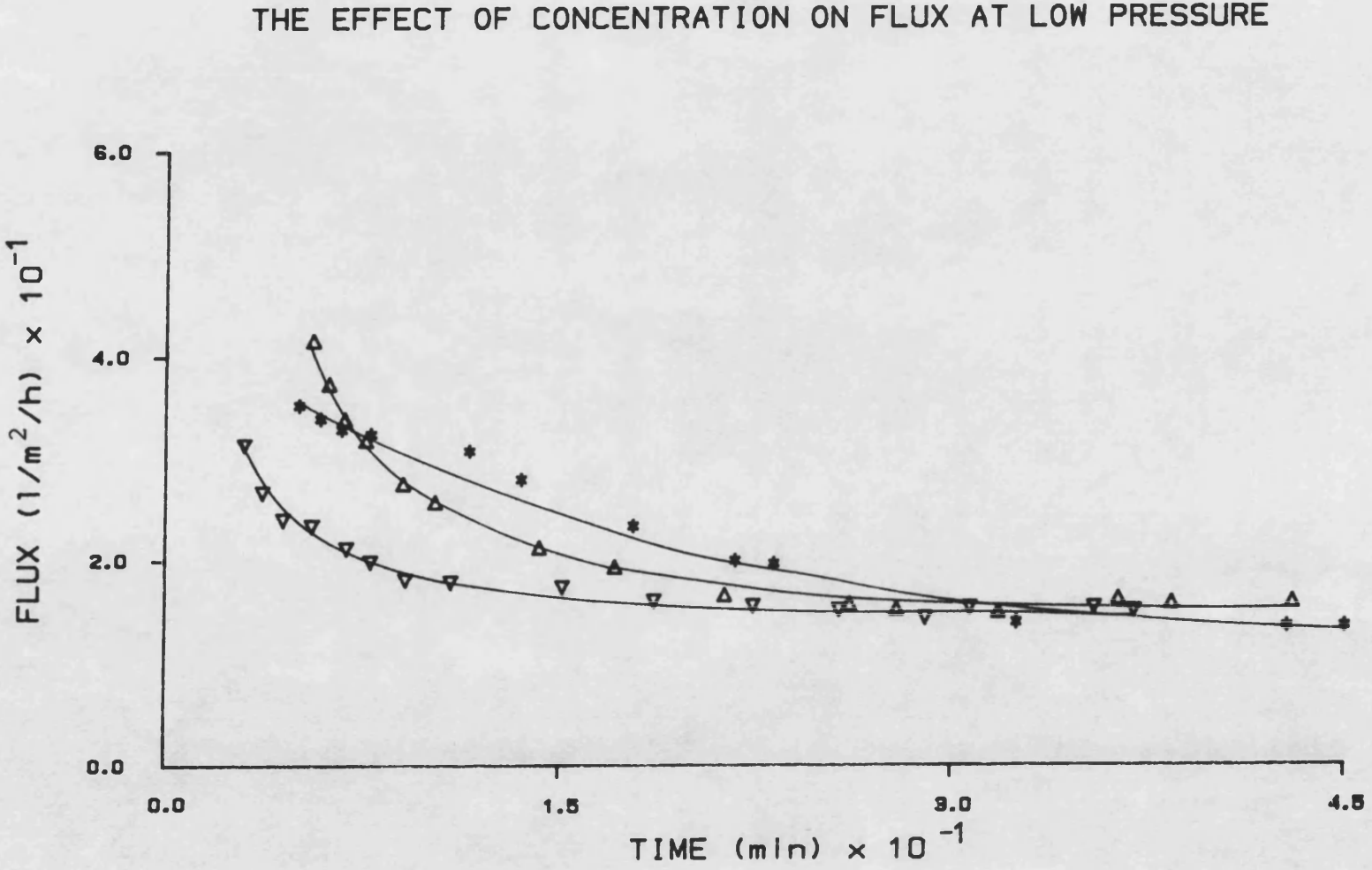


Fig.46: CFP-2E-3 Membrane; Recycling Flow Rate=0.5 ℓ/min; ΔP=1130Pa;T=10°C;
Feed: Whey Protein; 0.1 M Potassium Phosphate; pH=8.5;
* 5 g/ℓ; Δ 9.5 g/ℓ; ▽ 14.5 g/ℓ;

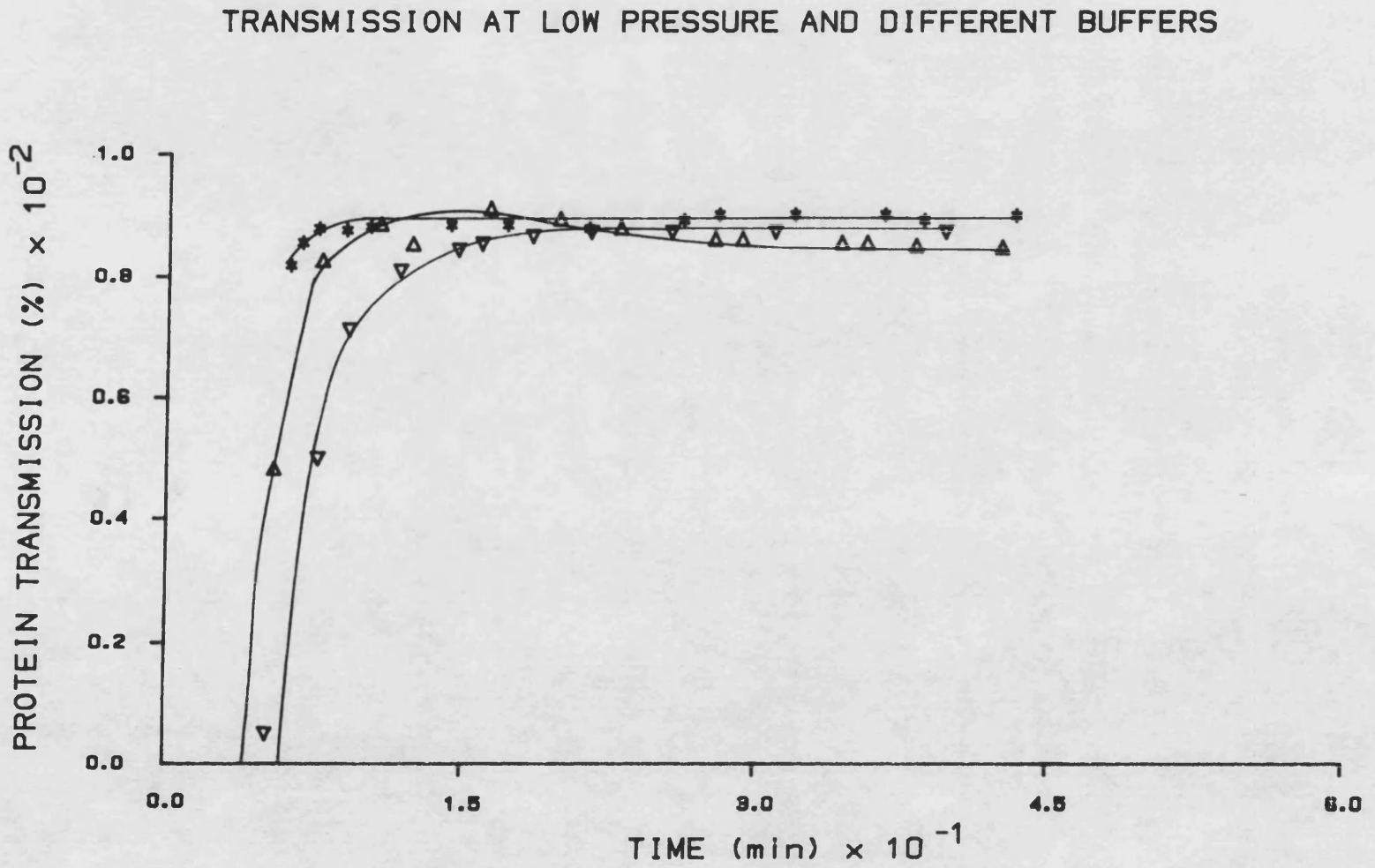


Fig.47: CFP-2E-3 Membrane; Recycle Flow Rate=0.5 ℓ/min; ΔP=1130Pa;T=10°C;
Feed: Whey Proteins; pH=8.5;* C_b=9.5 g/ℓ, 0.1 M Potassium Phosphate
Δ C_b=9.5 g/ℓ, 0.1 M Tris; ▽ C_b=8 g/ℓ, 0.1 M NaCℓ;

comparable whey protein solution obtained at higher pressure was 60 ℓ/m²/h and might very well be a result of a lower feed concentration.

More concentrated feed solutions yielded a high protein transmission too when subjected to a low operating pressure although a 10% rejection was detected (Fig.45). Fig.46 shows the transmembrane permeability of proteinaceous solutions at different concentrations. Flux seems to be independent of the feed concentration at such a low operating pressure. Fig.47 illustrates the effect of different buffer systems on protein transmission. Whey proteins dissolved in phosphate buffer transmitted at 95%, in NaCℓ buffer at 90% and in Tris buffer at 85%.

3. DISCUSSION

The results presented in sections 1 and 2 demonstrate clearly that protein transmission is a function of the transmembrane pressure. Transmission increases with decreasing operating pressure. The assumption that the transmission decline is a consequence of membrane compression as Goldsmith (1971) claimed would seem to be less appropriate here owing to the much lower pressures applied. Goldsmith deduced his theory from experiments performed in a pressure region of 1 - 2 MPa where a compaction of the membrane might occur, but the maximum pressure used in this work was with 20 kPa - two orders of magnitude lower.

It seems to be more likely that Spiegler and Kedem's (1966) suggestion of a predominant diffusion process at low pressures is the cause of the enhanced transmission. But since Spiegler and Kedem developed their theory from ultrafiltration experiments where the diffusion process might contribute substantially to the solute transport, it is doubtful if it fully applies to microporous membranes. The lag phase initially detected might be an indication of Spiegler and Kedem's theory. The slow transmission increase could be a consequence of the slower solute transport by diffusion compared to the more rapidly occurring transport by convection. Increased transmission at low pressure is perhaps simply a result of fewer particles arriving at the membrane surface at a time so that pore jamming is largely excluded. The observation of increased rejection at higher feed concentration could support this assumption. At higher protein concentration more particles arrive at the surface at a time so that a condition of spatial hindrance again arises. The increased protein transmission at low operating pressure after adding salt is most likely a result of the double layer compaction as discussed in Part III.

Part IV

Cell Harvesting and Cell Debris Removal

Molecular biology and recombinant DNA technology have provided methods in manipulating easily grown microorganisms such as Escherichia coli to produce proteins of interest by biosynthesis. This has led to a rising tendency to consider a fermentation producing biochemicals and pharmaceuticals. This has brought about an increasing demand for improved recovery technology. The often highly valuable and usually labile product requires careful handling to avoid unnecessary loss. Further important aspects are running costs and scale-up.

In cases of intracellular products the fermentation broth requires concentration prior to further treatment. Concentration techniques such as dead-end filtration and settling are tedious, labour-intensive and not always efficient. Centrifugation is likely to disintegrate the product and is often expensive both in capital and running costs. Cross-flow microfiltration devices offer a promising alternative to the traditional concentration techniques.

Following concentration the cells are ruptured and cell debris has to be removed. Intracellular product recovery is a difficult separation that has to be performed before the clarified extract can be subjected to more sophisticated purification steps. Cell debris separation by centrifugation can be troublesome due to lack of distinct density difference between the cell fragments and the media. Centrifugation also tends to produce aerosols. Cross-flow microfiltration offers an alternative to recover intracellular enzymes. Part IV is on the application of cross-flow microfiltration in recovery of intracellular products with main emphasis on cell debris removal.

1. Cell Harvesting

1.1 The Effect of Different Pore Sizes on Flux

Fig.48 shows the flux versus time profile for three cellulose acetate membranes with different nominal pore sizes. Flux declines sharply at the beginning and then levels off to a steady state value. It is somewhat surprising that the 0.6 μm membrane shows only

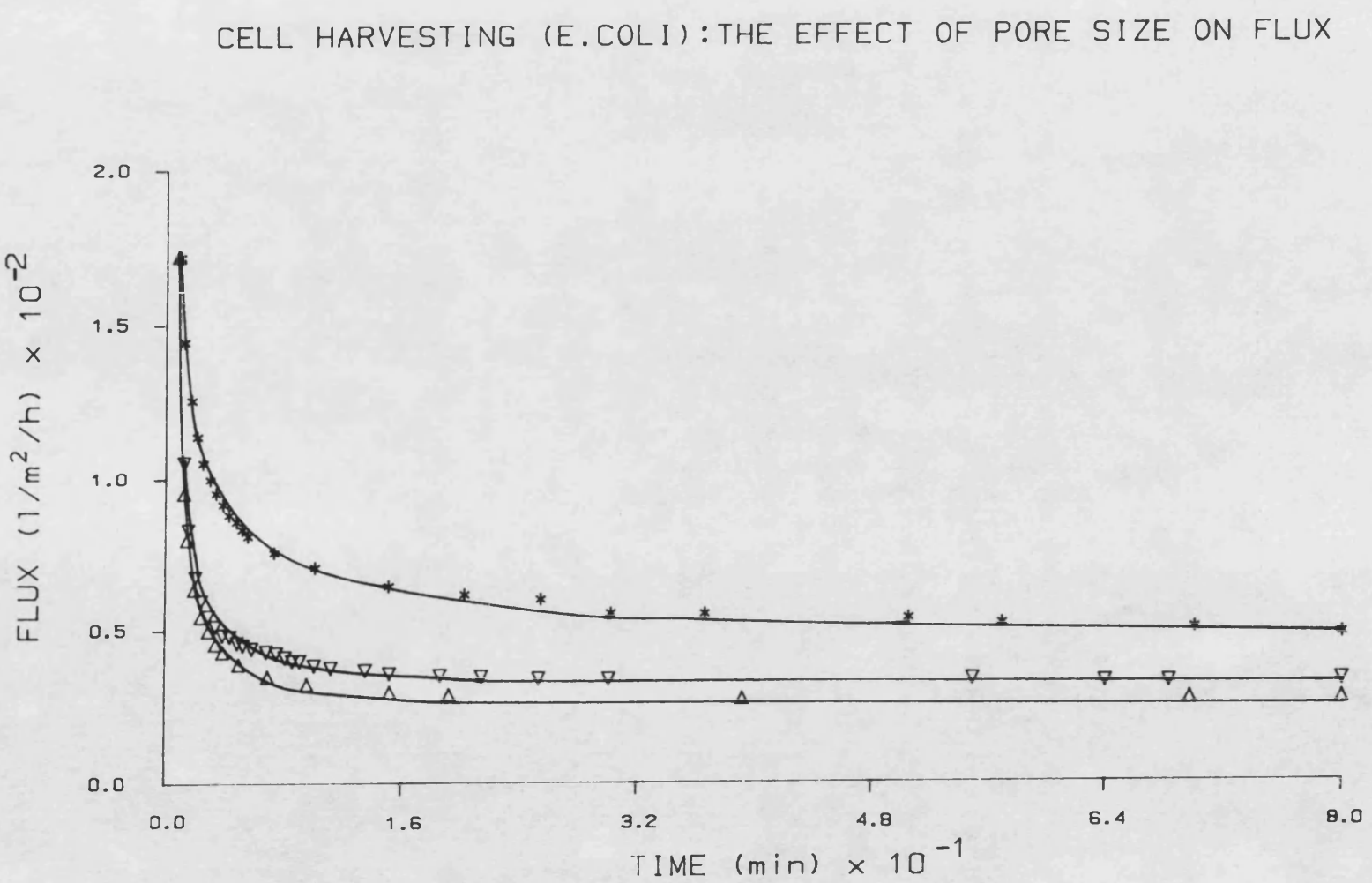


Fig.48: Asypor™ Membrane; ΔP=96 kPa; Recycle Flow Rate=1.6 ℓ/min; T=10°C;
Feed: E.coli; C_b=1.2 g/ℓ; pH=7;
Δ 0.2 μm; * 0.45 μm; ▽=0.6 μm;

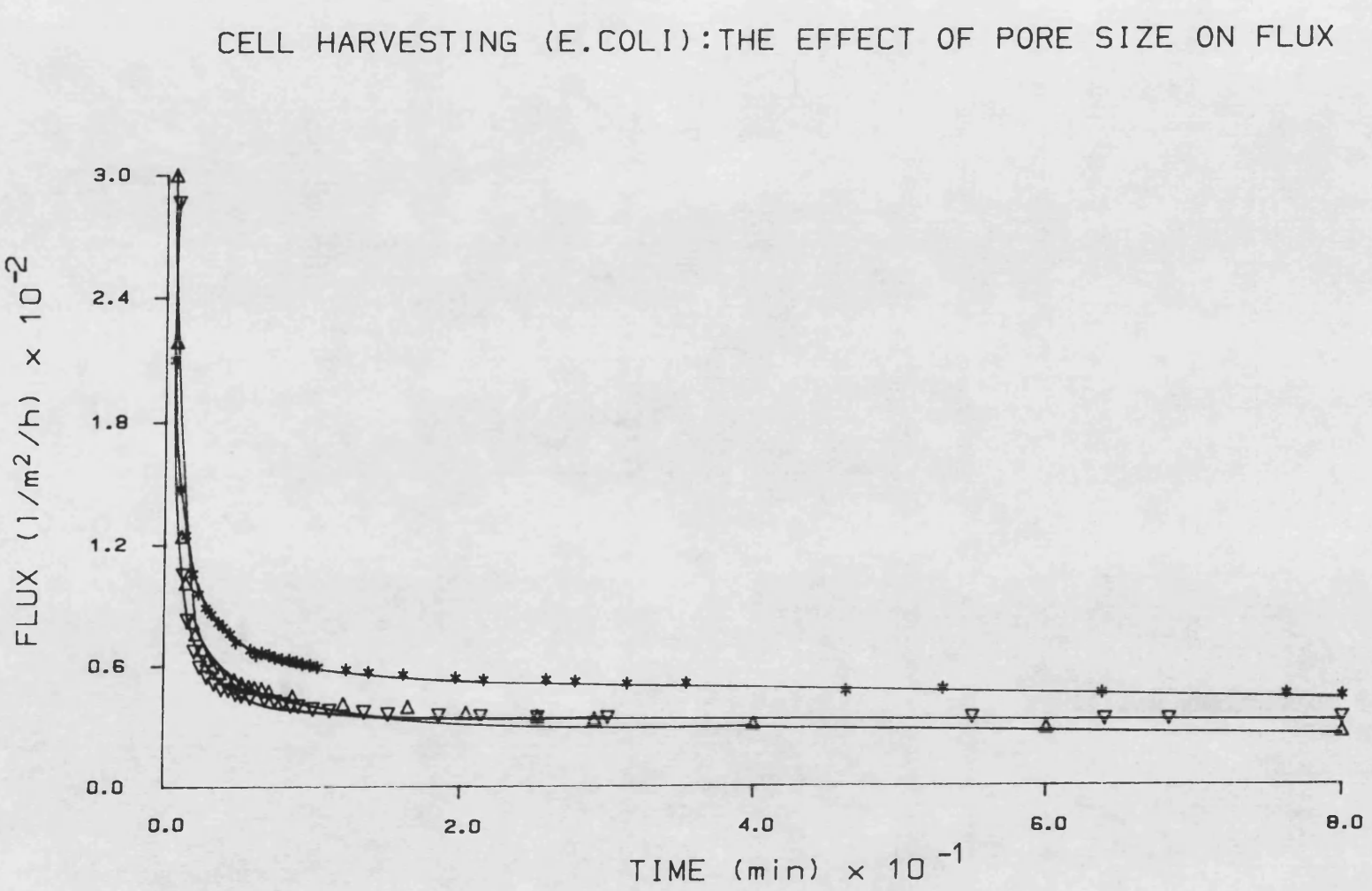


Fig.49: Nypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C;
Feed: E.coli; C_b=0.8 g/ℓ; pH=7;
Δ 0.2 μm; * 0.45 μm; ▽ 0.6 μm;

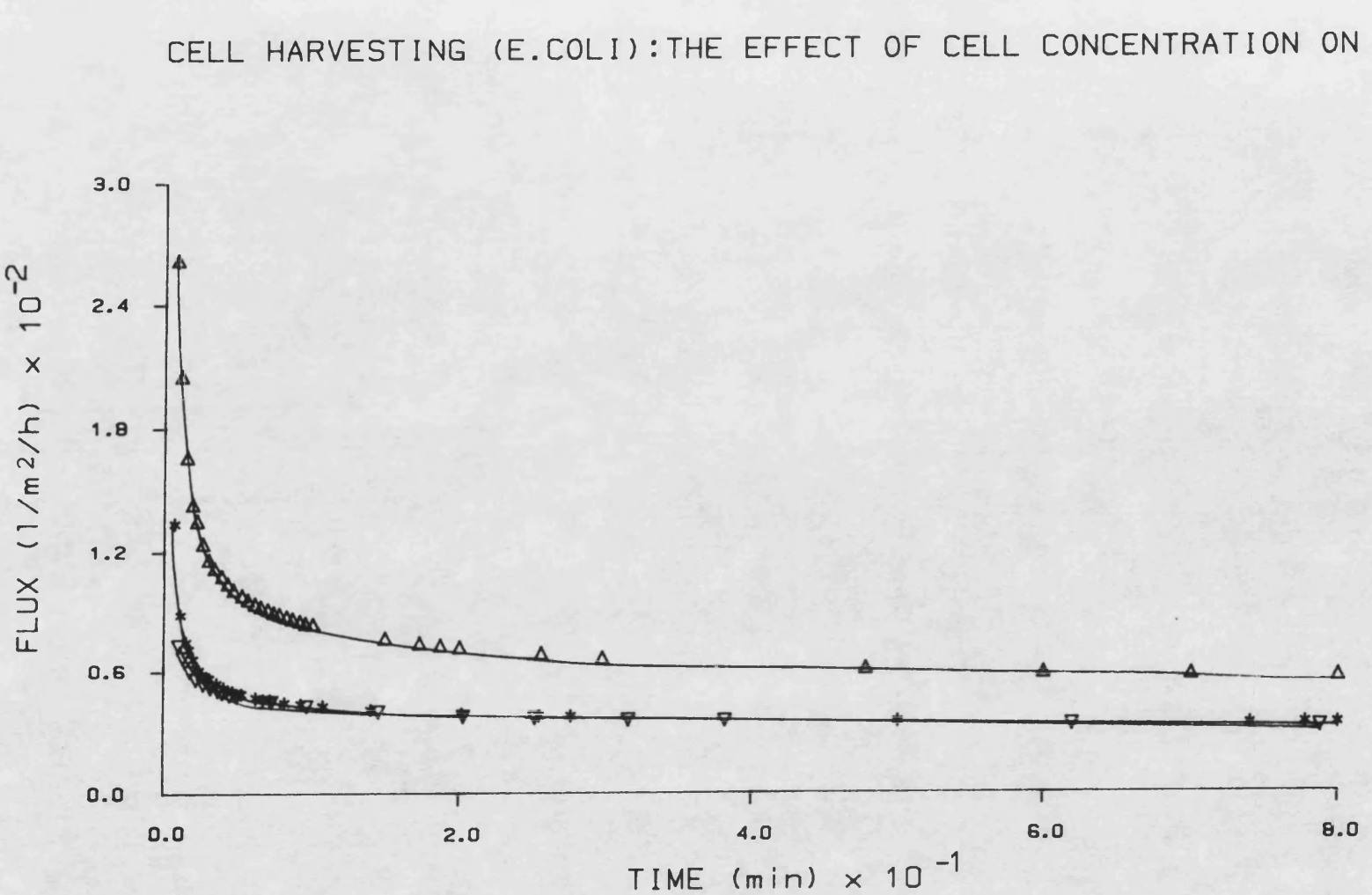


Fig.50: Nypor™ Membrane; (0.2 μm); ΔP=110 kPa; Recycle Flow Rate=1.5 ℓ/min
Feed: E.coli; pH=7; T=10°C;
Δ C_b=0.8; * C_b=1.4; ▽ C_b=2.1all in

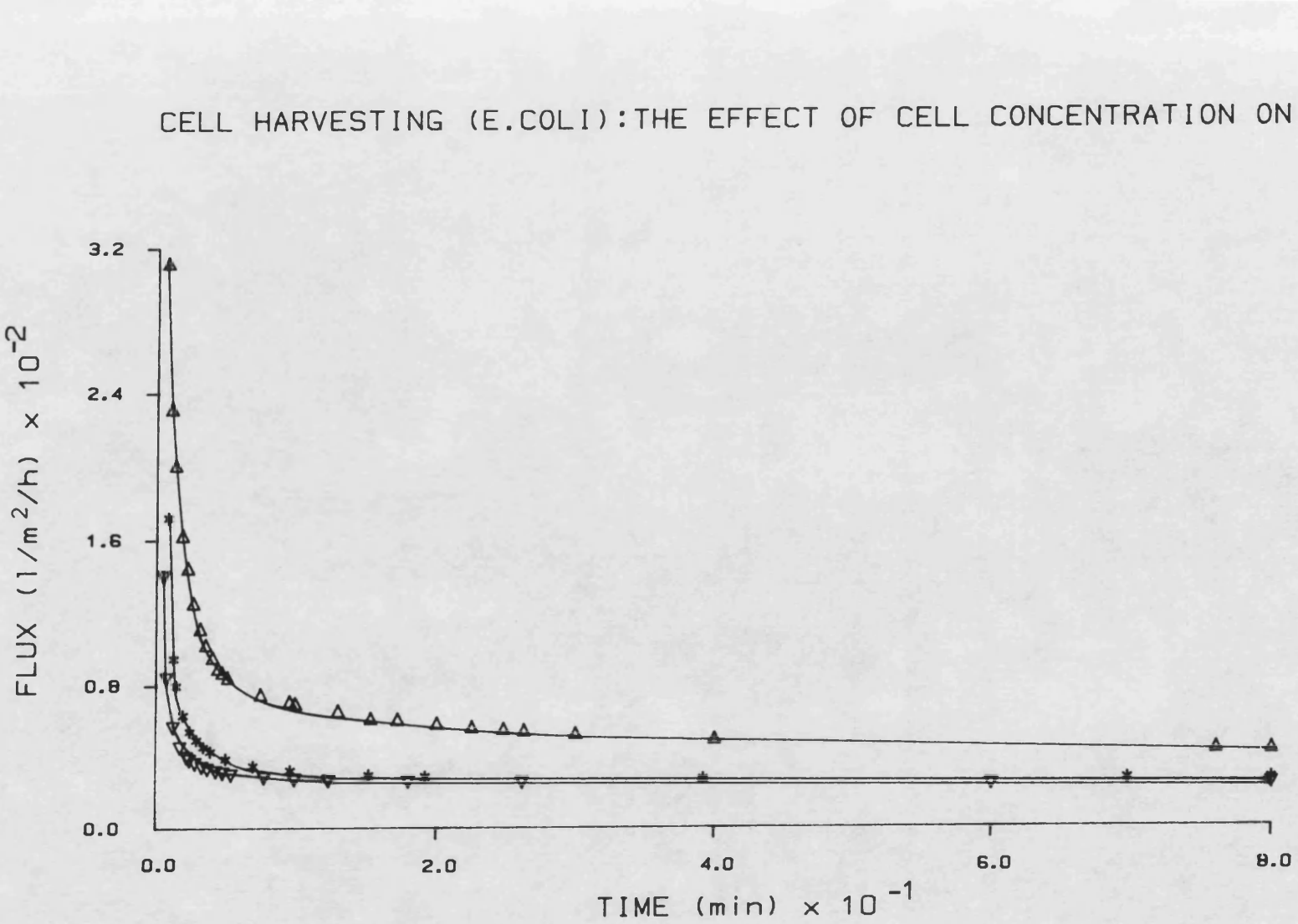


Fig.51: Asypor™ Membrane; (0.2 μm); ΔP=96 kPa; Recycle Flow Rate=1.7 ℓ/min
Feed: E.coli; pH=7; T=10°C;
Δ C_b=0.8; * C_b=1.2; ∇ C_b=1.8 all in

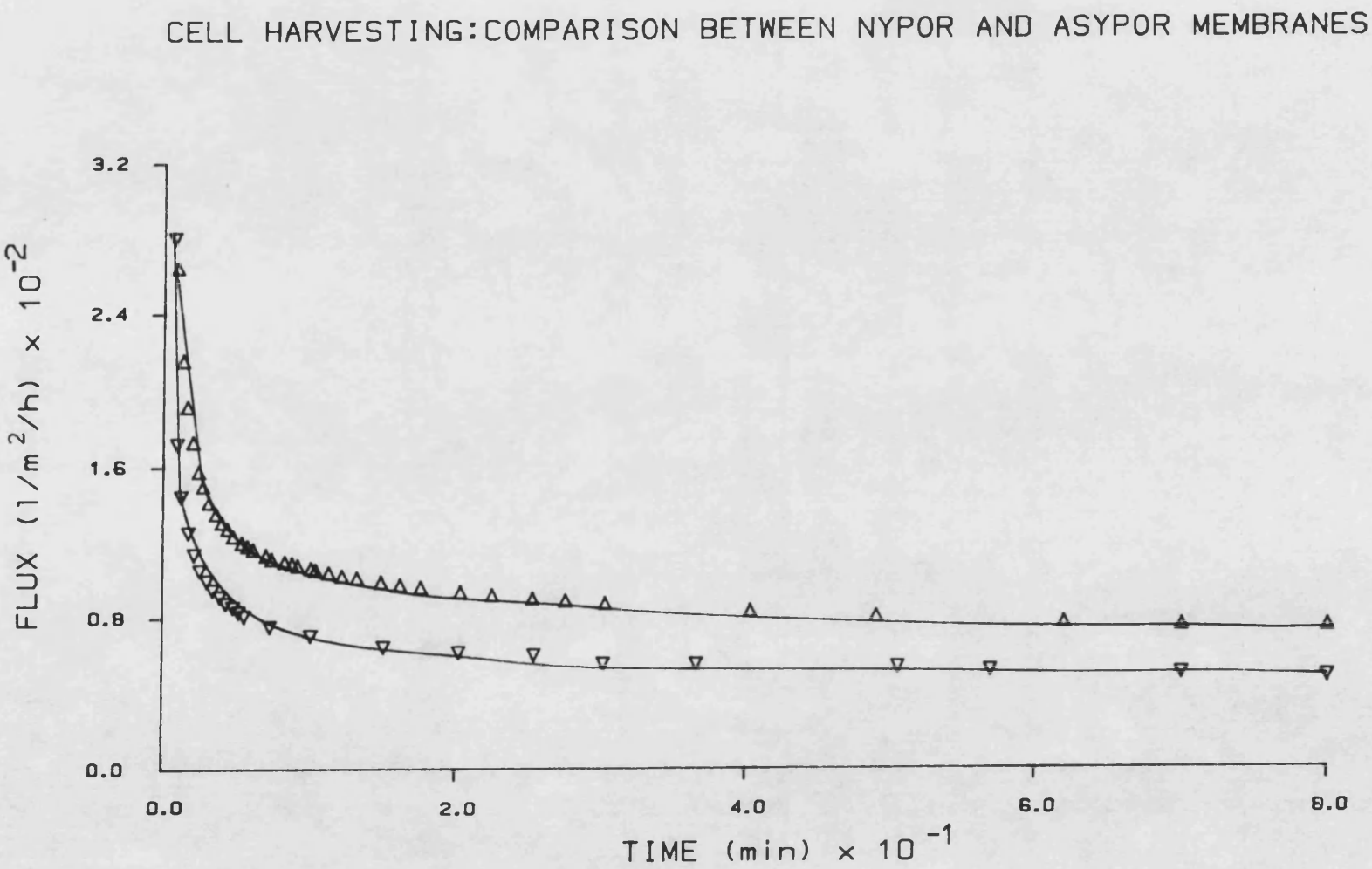


Fig.52: Feed: E.coli; C_b=1.2 g/ℓ; pH=7; T=10°C; ΔP=96 kPa;
Recycle Flow Rate=1.6 ℓ/min; Pore size=0.45 μm;
Δ Nypor™ Membrane; ▽ Asypor™ Membrane;

different nominal pore sizes. Flux declines sharply at the beginning and then levels off to a steady state value. It is somewhat surprising that the 0.6 μm membrane shows only 18% more flux than the 0.2 μm membrane, whilst the 0.45 μm membrane yielded almost twice the flux through the 0.6 μm membrane.
A qualitatively similar result was obtained with nylon membranes (Fig.49). In this case the operating pressure was low and thus so was the transmembrane flux. The difference between the membranes with respect to pore sizes was less pronounced than in the case of cellulose acetate membranes while once again the 0.45 μm membrane produced the highest flux.

Figs.50 and 51 demonstrate the effect of cell concentration on transmembrane permeability of a Nypor™ membrane and an Asypor™ membrane respectively. Both membranes show a reduced flux at higher broth concentrations. However, flux does not appear to be continuously dependent on the feed concentration but seems to remain constant once a certain concentration is exceeded.

Since both membranes consist of different materials it was interesting to see if they differed in permeability when exposed to the same proteinaceous suspension. The results with respect to different pore sizes are listed below:

Permeability of Asypor™ and Nypor™ Membranes in [ℓ/m²/h]

Type of Membrane	Pore Size 0.2 μm	0.45 μm	0.6 μm
Asypor™	44 (ΔP=110kPa)	57 (ΔP=96kPa)	32 (ΔP=14kPa)
Nypor™	57 (ΔP=110kPa)	86 (ΔP=96kPa)	32 (ΔP=14kPa)

There was no difference in flux noticed with a pore size of 0.6 μm whilst with a pore size of 0.2 μm the Nypor™ membrane had a 30% higher flux than the Asypor™ membrane and a 50% higher flux when the pore size was of 0.45 μm (Fig.52).

Le and Atkinson (1985) made qualitatively similar observations of flux with respect to pore size and attributed the effect to the variation between the ratio of the bacterial cell diameter and the pore diameter. They suggested that with a ratio close to unity pore bridging will be more probable and the resulting flux is that through freely permeable and protected pores. Thus flux through a 0.45 μm is highest. In the case with a pore size bigger than the particle size pores are more likely to become blocked and flux is reduced. If the pores are very small as with 0.2 μm membranes permeation is determined by the rate at which cells are dislodged either by back-diffusion, or more likely when they are

swept away by eddies in the fluid flowing across the membrane.

Furthermore the flux time profiles for both the 0.45 μm Aspor™ and Nypor™ membranes, in contrast to those for the others, showed a further flux decline with elapsing time. This gives evidence for the bridging theory. A growing number of pores become blocked and simultaneously fewer pores are available to the bridging process.

The observation that flux becomes independent of the feed concentration above a certain concentration differs from the observations made in ultrafiltration. It is quite likely that at a certain concentration the rate at which the pores are cleaned by eddies is counter-balanced by the rate at which new particles are brought to the membrane. Flux is then entirely a function of hydrodynamics and independent on the feed concentration.

The higher flux in the case with Nypor™ membranes of 0.2 μm and 0.45 μm compared to Aspor™ membranes might be due to different solid-membrane interactions because of different membrane materials. Nylon appears to interact less with the proteinaceous material and produces a higher flux.

2. Cell Debris Removal

2.1 β-galactosidase

This set of experiments was designed in the main to achieve maximum enzyme transmission. Previous investigations showed that protein transmission was highest when the proteinaceous material was dissolved in a potassium phosphate buffer and the transmembrane pressure was as low as possible. Consequently it was decided to operate the system at a low pressure accepting a poor flux regardless of the fact that this is one of the most important criteria and very often equated with system performance.

Figs.53 and 54 confirm the prediction of a low transmembrane permeability. The Aspor™ and Nypor™ membranes of different pore size show a flux around 20 $\ell/m^2/h$.

Figs.55 and 56 illustrate the total protein transmission observed when disrupted E.coli was filtered through Nypor™ and Aspor™ membranes of different pore sizes. The transmission of β-galactosidase through the two types of membranes is shown in Figs.57

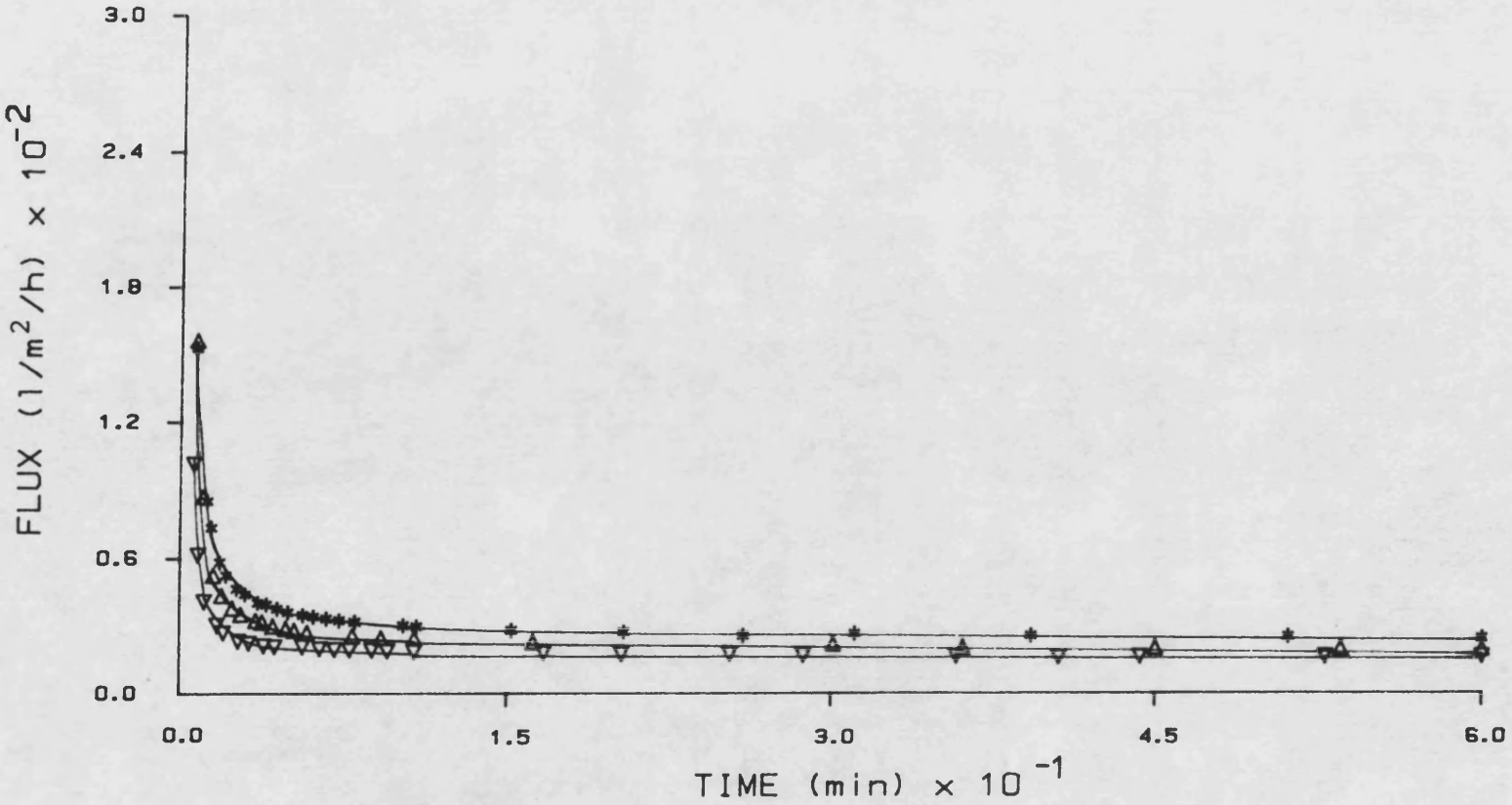


Fig.53: Nypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C;
Feed: Resuspended E.coli; C_b=2.5 g/ℓ ;pH=7;
Δ 0.2 μm; * 0.45 μm; ∇ 0.6 μm;

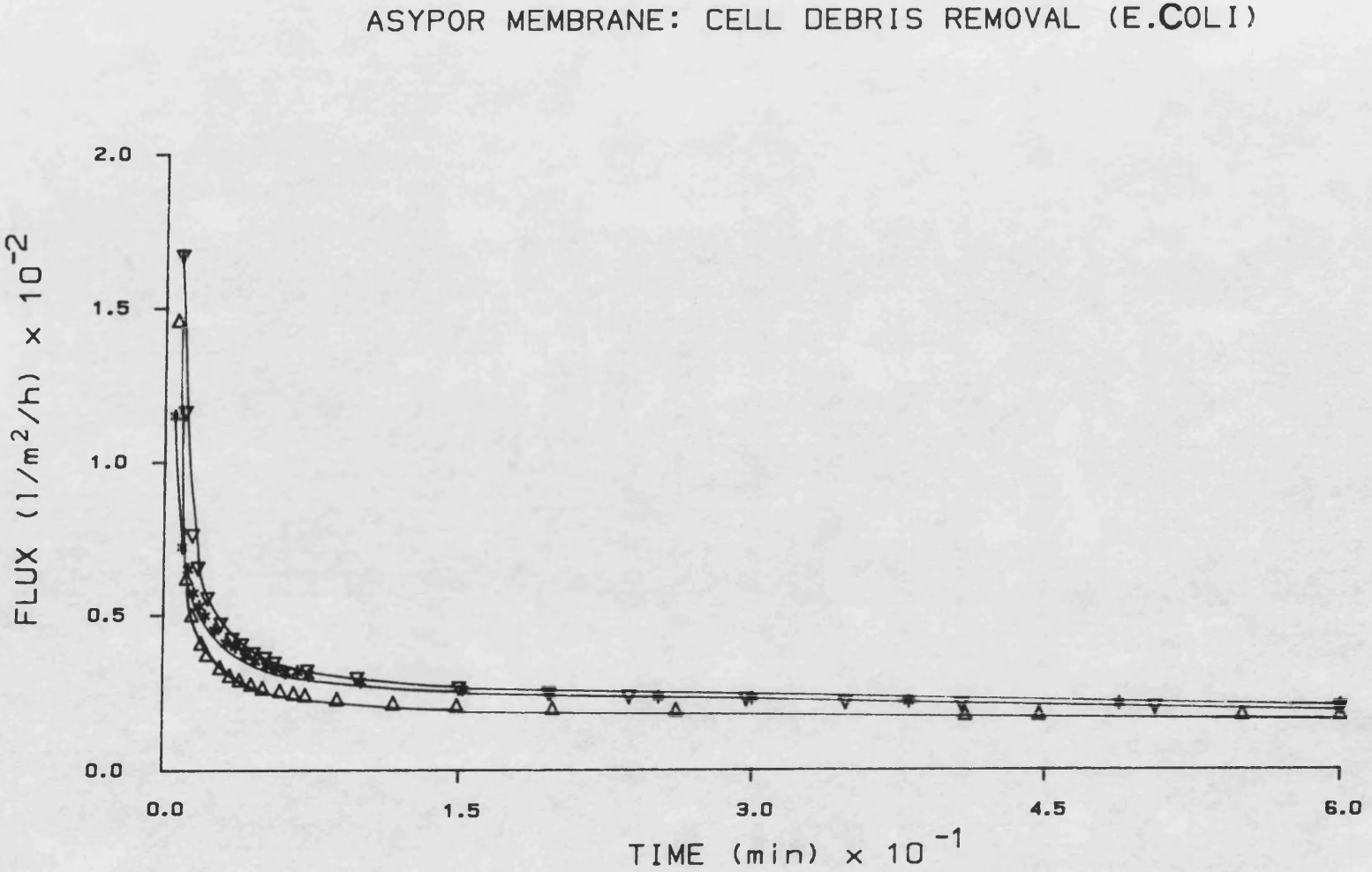


Fig.54: Asypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C;
Feed: Resuspended E.coli; C_b=2.5 g/ℓ ; pH=7;
Δ 0.2 μm; * 0.45 μm; ▽ 0.6 μm;

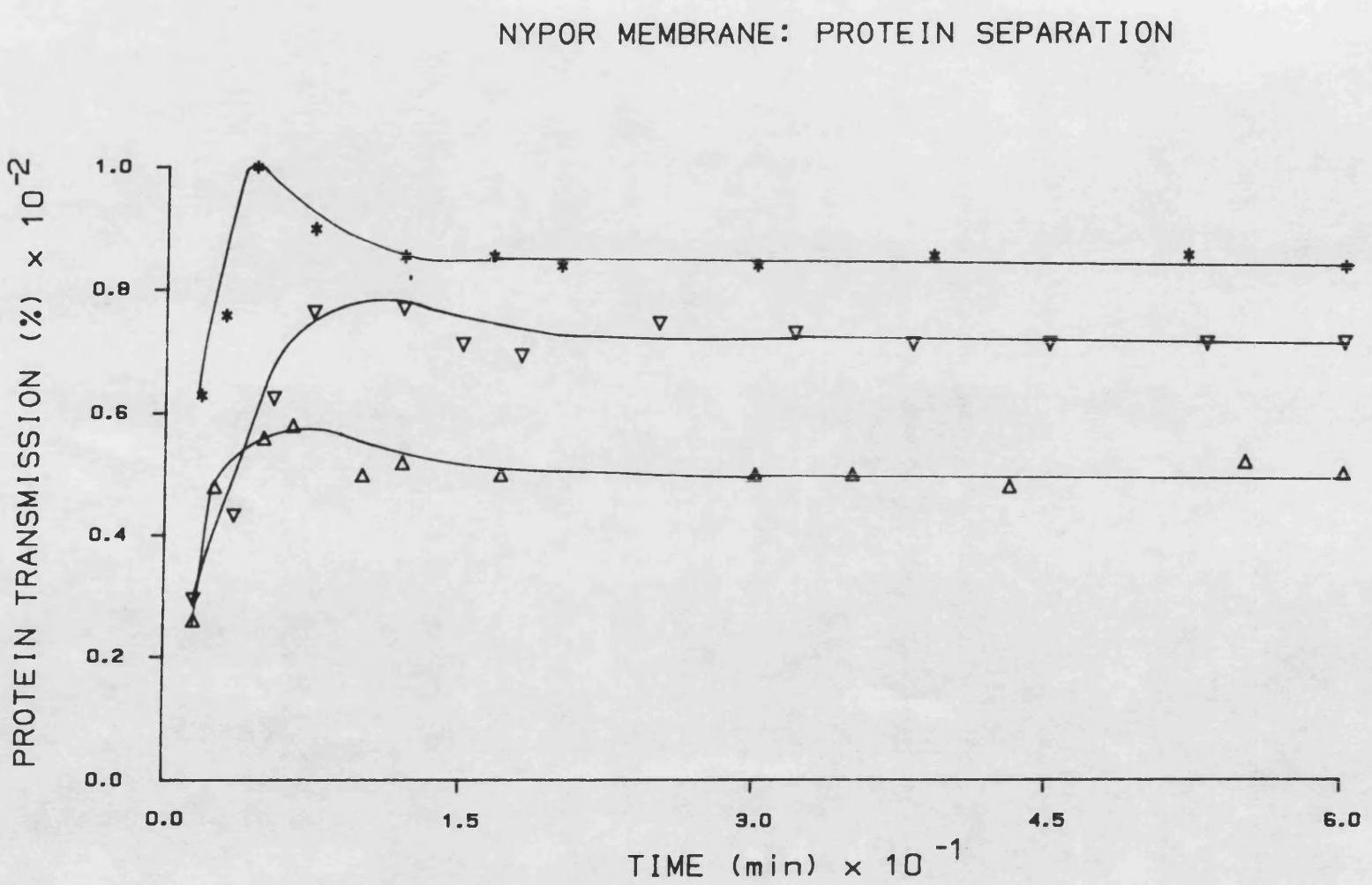


Fig.55: Nypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C;
Feed: Resuspended E.coli; C_b=2.5 g/ℓ ;pH=7;
Δ 0.2 μm; * 0.45 μm; ▽ 0.6 μm;

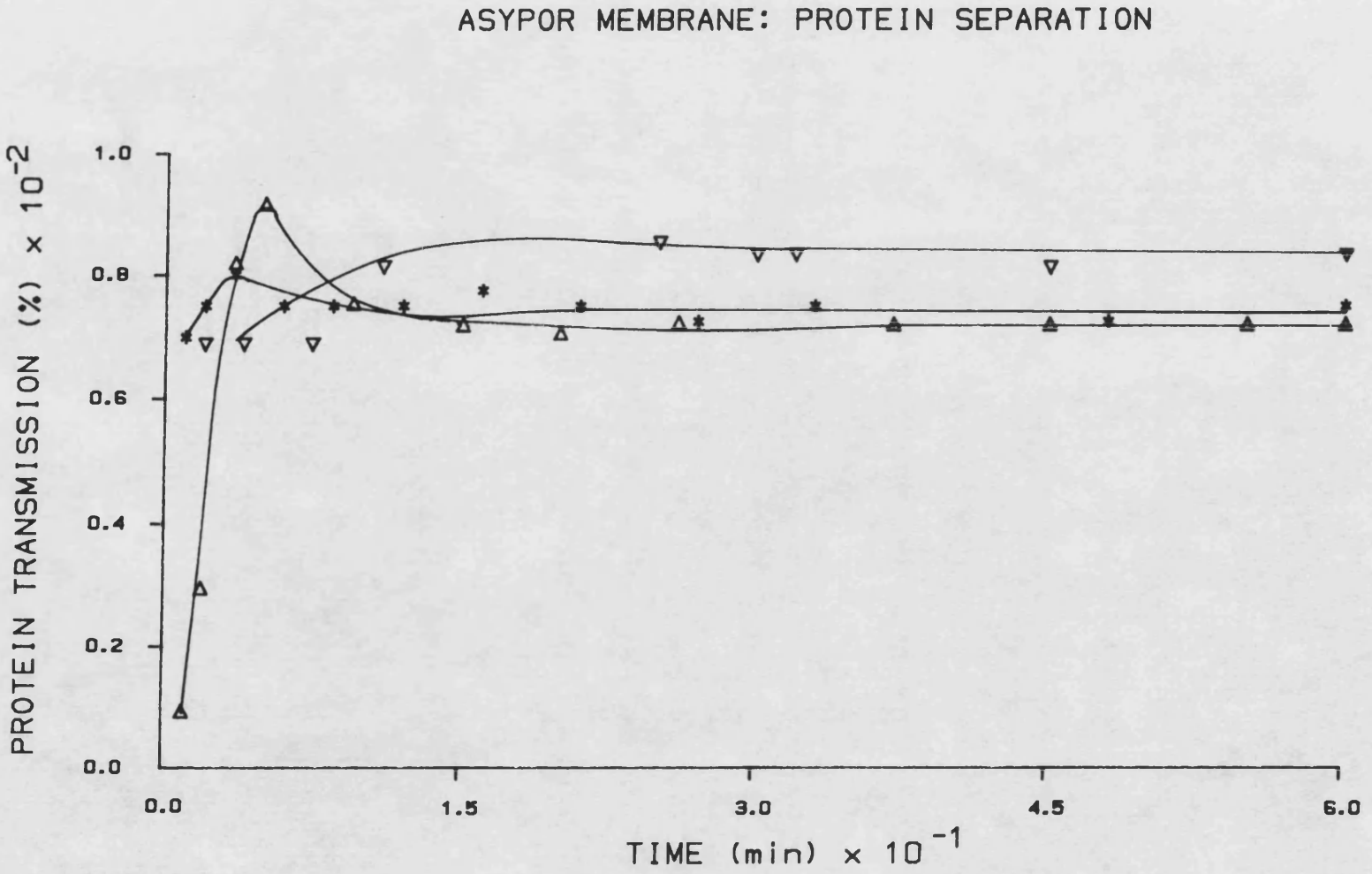


Fig.56: Aspor^{TM} Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10^{0}C;
Feed: Resuspended E.coli; C_b=2.5 g/ℓ ; pH=7;
Δ 0.2 μm; * 0.45 μm; ∇ 0.6 μm;

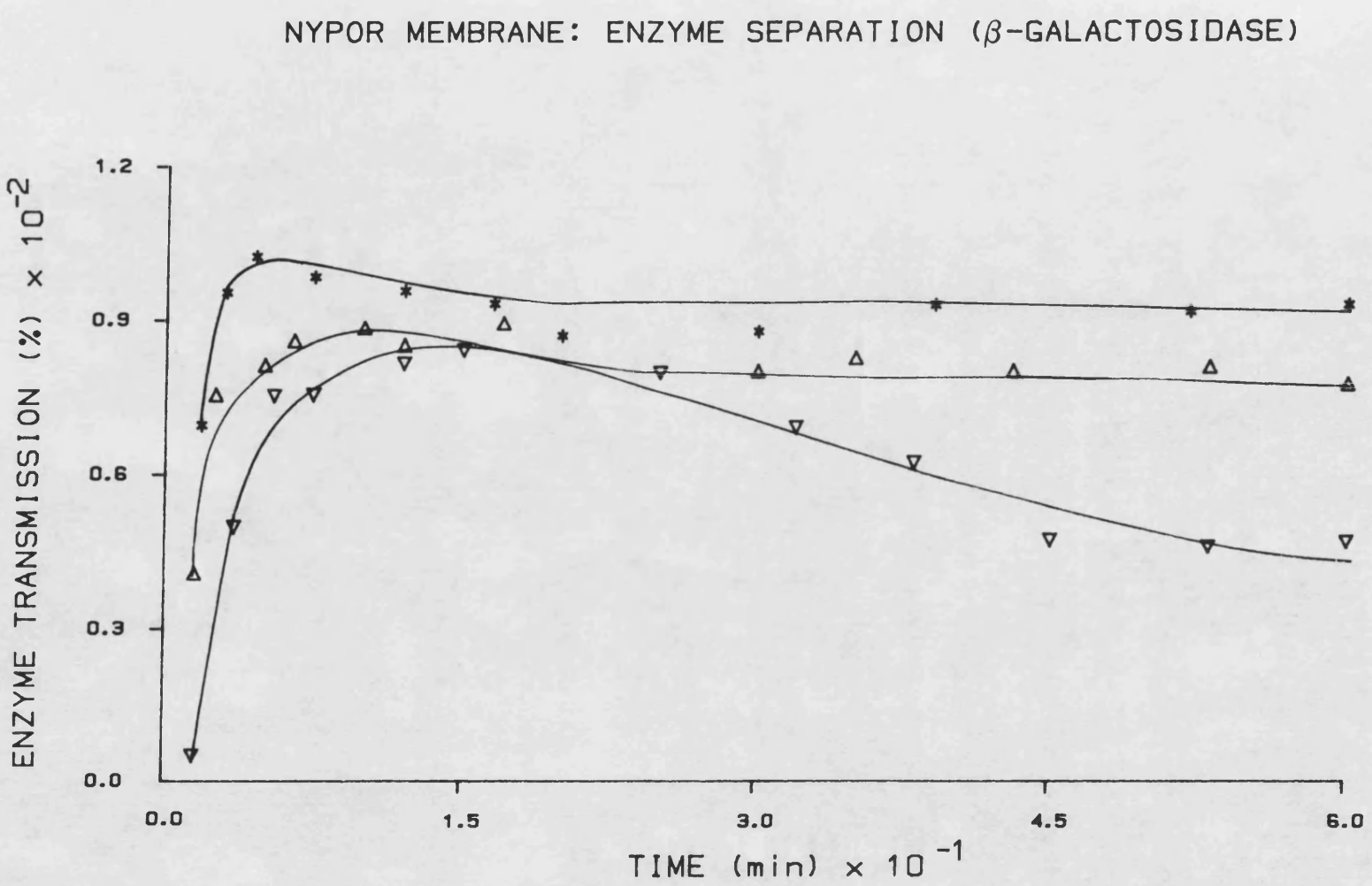


Fig.57: Nypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C;
Feed: Resuspended E.coli; C_b=2.5 g/ℓ ; pH=7;
Δ 0.2 μm; * 0.45 μm; ▽ 0.6 μm;

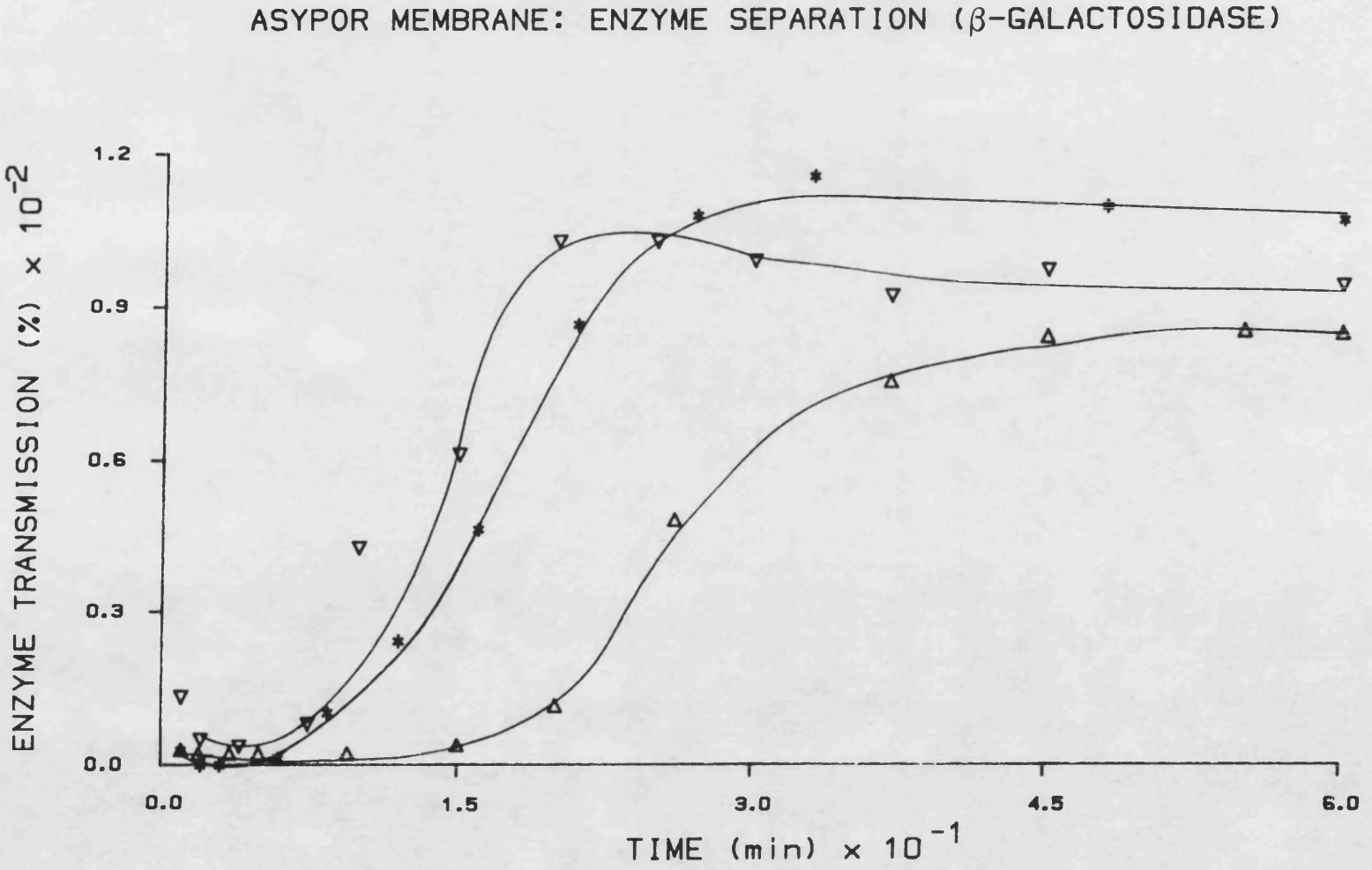


Fig.58: Asypor™ Membrane; ΔP=14 kPa; Recycle Flow Rate=0.6 ℓ/min; T=10°C; Feed. Resuspended E.coli; C_b=2.5g/1; pH=7; Δ 0.2μm; * 0.45μm; ∇ 0.6μm;

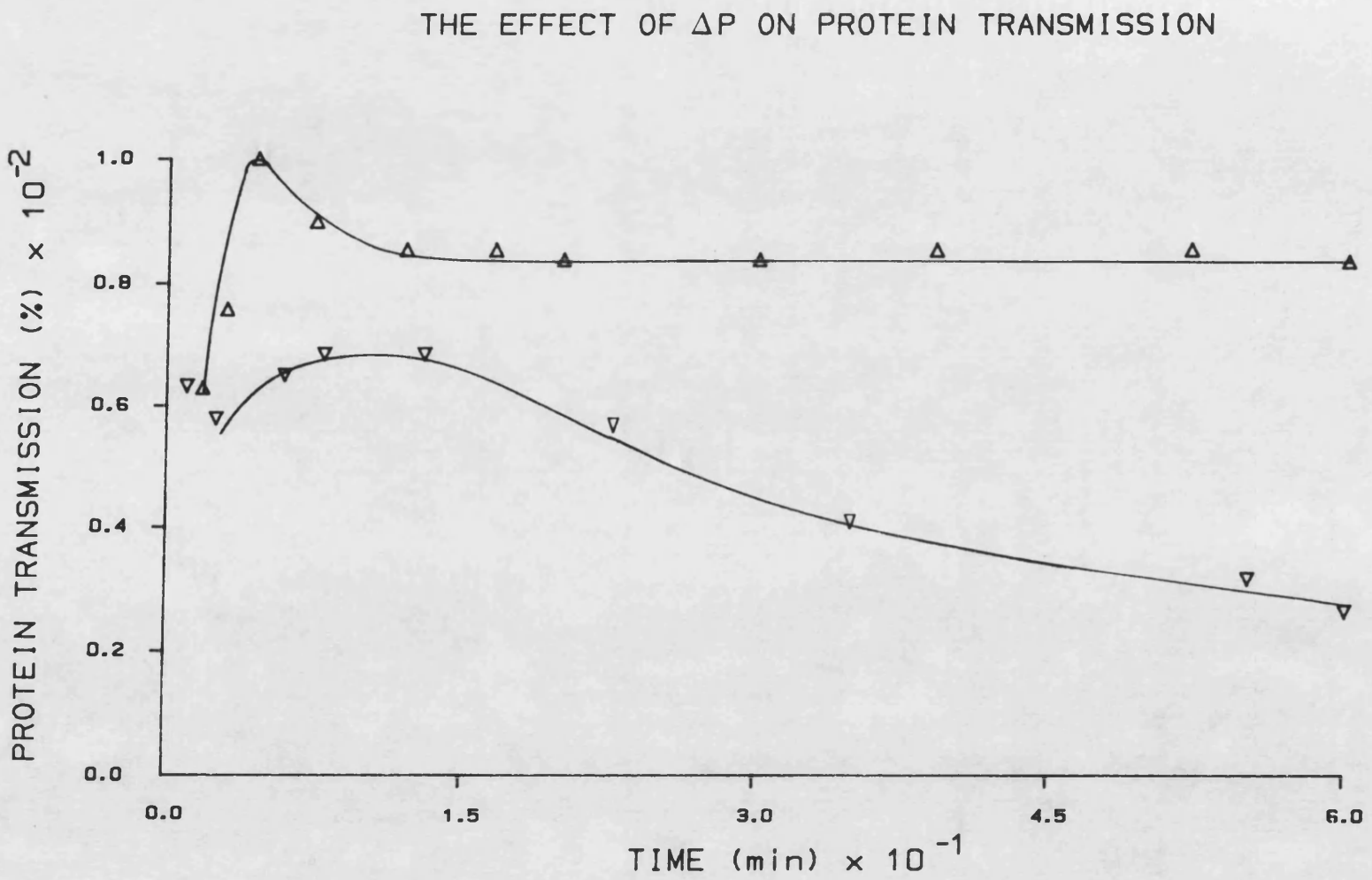


Fig.59: Nypor™ Membrane (0.45 μm); Feed: Resuspended E.Coli; T=10°C;
C_b=2.5 g/ℓ; pH=7; Δ ΔP=14 kPa, Recycle Flow Rate=0.6 ℓ/min;
▽ ΔP=70 kPa, Recycle Flow Rate=43 ℓ/min;

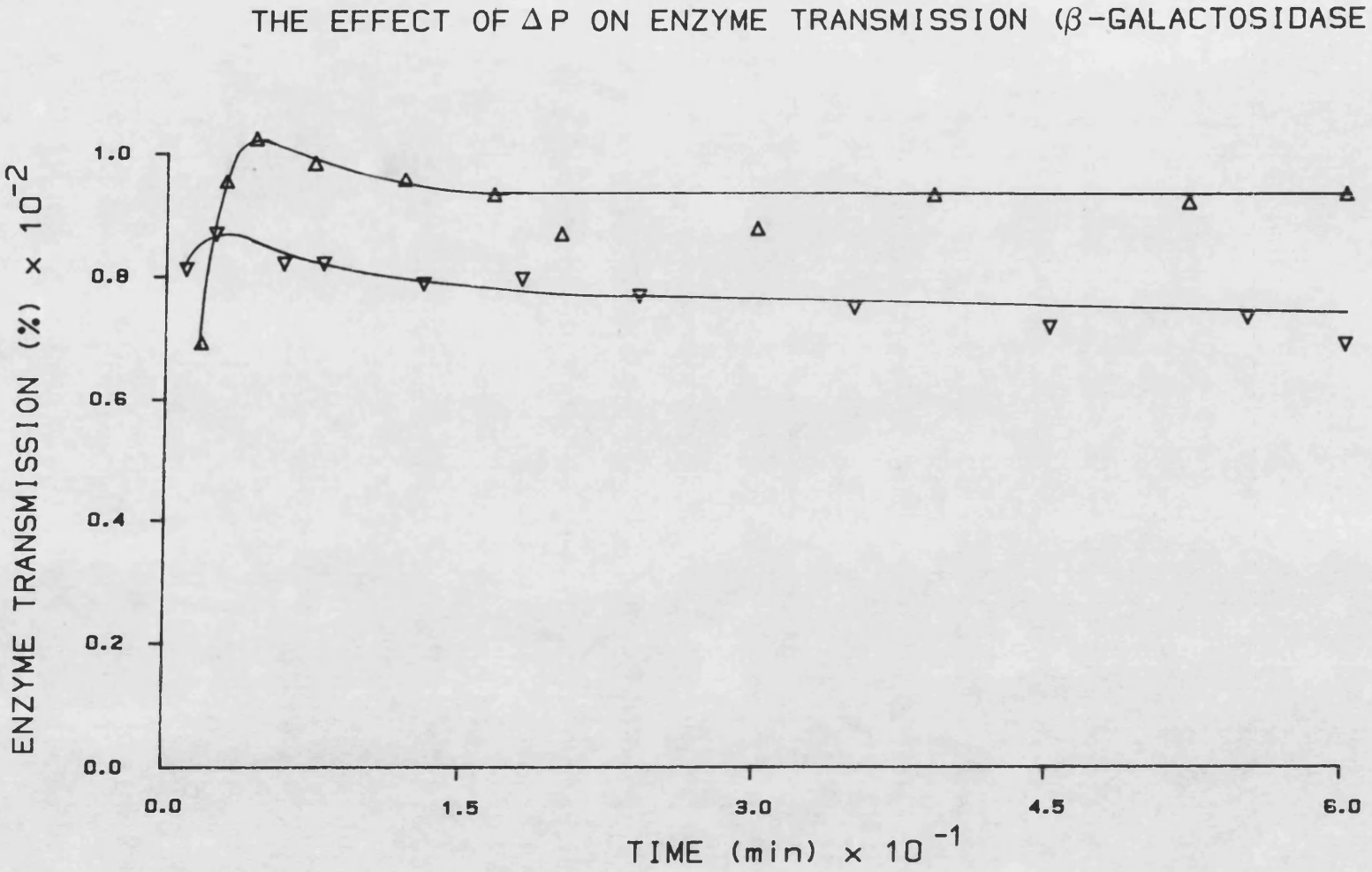


Fig.60: Nypor™ Membrane (0.45 μm); Feed: Resuspended E.Coli; T=10°C;
C_b=2.5 g/ℓ; pH=7; Δ ΔP=14 kPa, Recycle Flow Rate=0.6 ℓ/min;
∇ ΔP=70 kPa, Recycle Flow Rate=1.3 ℓ/min;

Figs.55 and 56 illustrate the total protein transmission observed when disrupted E.coli was filtered through Nypor^{TM} and Aspor^{TM} membranes of different pore sizes. The transmission of β-galactosidase through the two types of membranes is shown in Figs.57 and 58. Total protein transmission of the Nypor^{TM} membrane was at 50% lowest with a pore size of 0.2 μm, highest at 83% with 0.45 μm and at 71% with a 0.6 μm membrane. 0.45 μm was also the optimal pore size for β-galactosidase transmission with 93%, 0.2 μm showed a 10% less transmission whilst 0.6 μm rejected 50% of the product of interest. The results are listed below:

Total Protein -and β-Galactosidase Transmission through Nypor^{TM} Membranes

Pore Size (μm)	Total Protein Transmission (%)	β-Galactosidase Transmission (%)
0.2	50	83
0.45	83	93
0.6	71	50

Aspor^{TM} membranes exhibited an increasing total protein transmission with increasing nominal pores size and showed a less pronounced difference in the steady state values. Transmissions were 72% (0.2 μm), 75% (0.45 μm) and 88% (0.6 μm). β-galactosidase transmission was again highest at 0.45 μm (110%) reduced at 0.6 μm (95%) and lowest at 0.2 μm (77%). The results are listed below:

Total Protein -and β-Galactosidase Transmission through Aspor^{TM} Membranes

Pore Size (μm)	Total Protein Transmission (%)	β-Galactosidase Transmission (%)
0.2	72	77
0.45	75	110
0.6	88	95

The most striking feature of Fig.58 is the extremely retarded transmission over a relatively long initial period. This phenomenon was not observed in the case of total protein transmission and also not evident when Nypor^{TM} membranes were used. A comparison of transmission values between the two types of membranes yields that transmission was on the average higher for Aspor^{TM} membranes than for Nypor^{TM} membranes.

the average higher for Asypor^{TM} membranes than for Nypor^{TM} membranes.

Figs.59 and 60 confirm the assumption that a low transmembrane pressure would produce the highest total protein and β-galactosidase transmission. A high operating pressure depressed total protein transmission in particular, which declined to a value of 30% after 60 minutes and did not show any tendency to level off to a steady state.

The flux profiles of cell debris removal seem to be independent of membrane material and pore size unlike in the case of cell harvesting. Presumably this can be traced back to the composition of the cell debris feed. A suspension of lysed cells contains many different proteins, enzymes and DNA covering a whole range of molecular weights. Besides, ultrasonic treatment generates differently sized cell wall fragments. Hence pore jamming is likely to occur at least with 0.45 and 0.6 μm membranes perhaps also in the case of a 0.2 μm membrane. However, with a pore size of 0.2 μm flux will also be restricted by pore blockage because of deposited proteinaceous material on top of the membrane surface.

Particle transmission through the Asypor^{TM} membrane was on average higher than through the Nypor^{TM} membrane. It appears that cellulose acetate is less strongly charged hence transmission is less retarded. As discussed in Part I it is possible that the amide groups of the Nypor^{TM} membrane are polarised and induce a relatively strong negative charge which might repel proteins to some extent when they are above their isoelectric point.

Provided the assumption that Nypor^{TM} membranes interact less with proteins is valid the transmission profile should follow the relative particle-pore size theory. That implies for this particular membrane-protein system an optimal pore size of 0.45 μm which is confirmed in Fig.55 and 57. The low β-galactosidase transmission through a 0.65 μm membrane is presumably due to severe pore plugging hence the remaining accessible pore area is considerably reduced so that β-galactosidase with a molecular weight of 520,000 is increasingly rejected.

In the case of cellulose acetate membranes protein transmission is more determined by membrane-protein interaction than pore plugging. Since adsorption is less dependent on pore size the differences in the steady state values are less distinct. The high protein transmission at a pore size of 0.6 μm is attributable to small proteins which most likely make up a big portion of the transmitted fraction. It is furthermore assumed that small

proteins pass through first before β-galactosidase starts to transmit. This is supported by the behaviour of enzyme transmission. During a prolonged initial phase no transmission of β-galactosidase was detectable. Transmission occurred then with the order of pore sizes. At the steady state the transmission profile reflected once more the relative particle-pore size theory with 0.45 μm being the optimal pore size. A high transmembrane pressure appears to have a seriously deleterious effect on total protein and β-galactosidase transmission. At low pressure presumably less particles arrive at the membrane surface at a time and pore jamming is less likely.

2.2 Alcohol-Dehydrogenase

Yeast debris is one of the most difficult proteinaceous materials to process. Disrupted resuspended yeast cells produce a highly viscous suspension and the cell wall fragments are hydrophobic in nature and tend to be adhesive. These rheological properties indicate the problems which arise when membranes are used to separate an enzyme from yeast debris.

Alcohol dehydrogenase, an intracellularly produced enzyme of Bakers yeast was chosen to investigate a potential application of cross-flow microfiltration to yeast debris removal.

Fig.61 illustrates the flux obtained with two membranes of the same pore size but manufactured of different materials. The hydrophilic cellulose acetate membrane showed a higher permeability during the initial phase but approached the flux of a hydrophobic polytetrafluoroethylene membrane when time elapsed. The flux at 8 $\ell/m^2/h$ was extremely low. A poor filtration performance with respect to the rate of permeability for resuspended yeast debris was also observed by Le and Billiet (1984).

The result of similar flux for both membranes was somewhat unexpected. It was thought that the cellulose acetate membrane would yield a higher flux than the hydrophoic PTFE membrane since it is well known that membranes with hydrophobic properties are more prone to protein adsorption and fouling respectively than hydrophilic membranes. (Dillman and Miller, 1973; Matthiasson, 1985). However, PTFE is also lipophobic (Speaker, 1985) and may not interact hydrophobically with these proteins.

Presumably the predominant process of pore plugging in the case of a cell debris suspension described in the former section is the cause of the observed low membrane

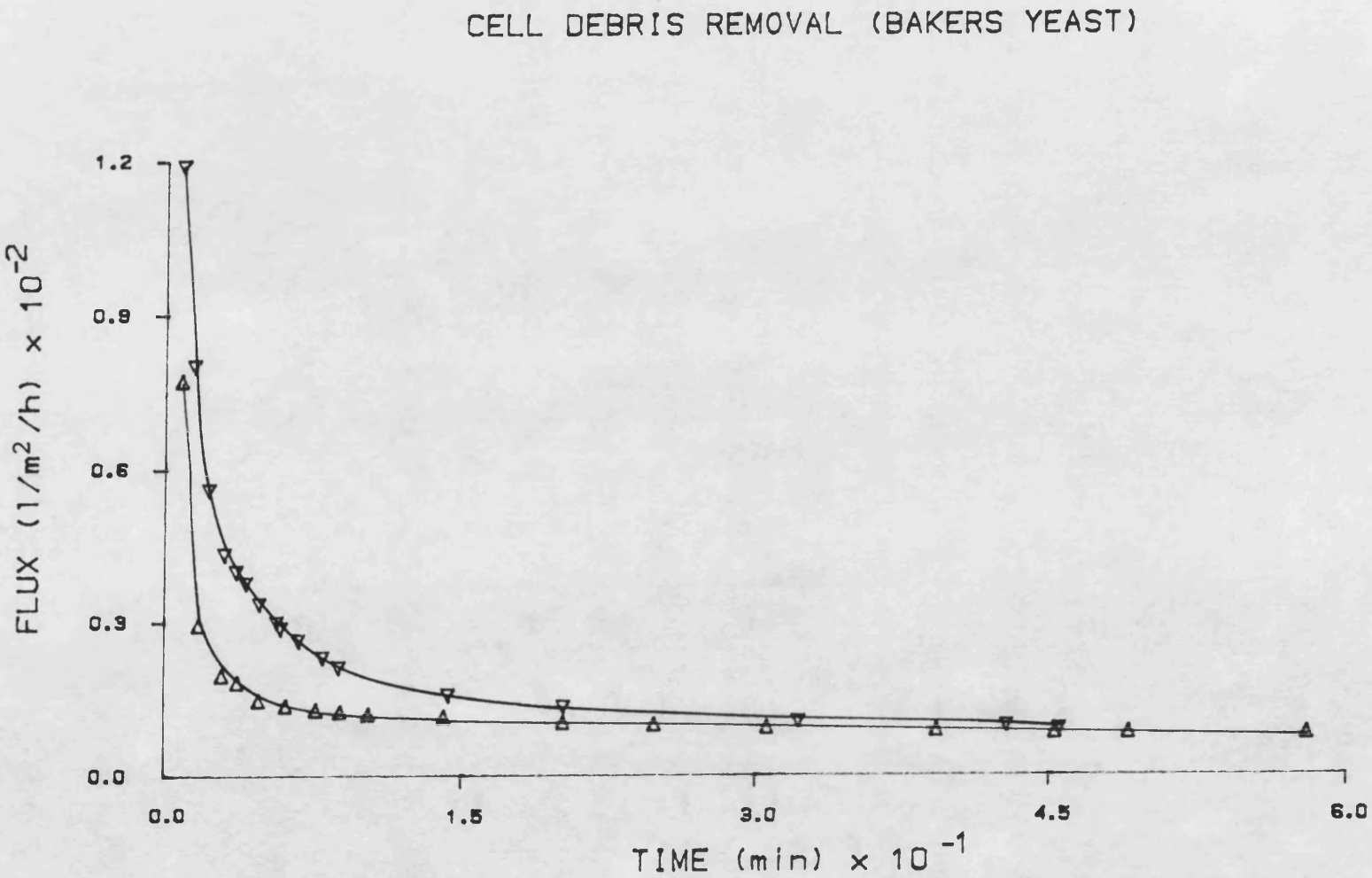


Fig.61: Feed: Bakers Yeast; 0.1 M Potassium Phosphate; pH-7.4; T-10°C; Pore size-0.45 μm; ΔP-14 kPa; Recycle Flow Rate-0.4 ℓ/min; Δ PTFE; ∇ CA;

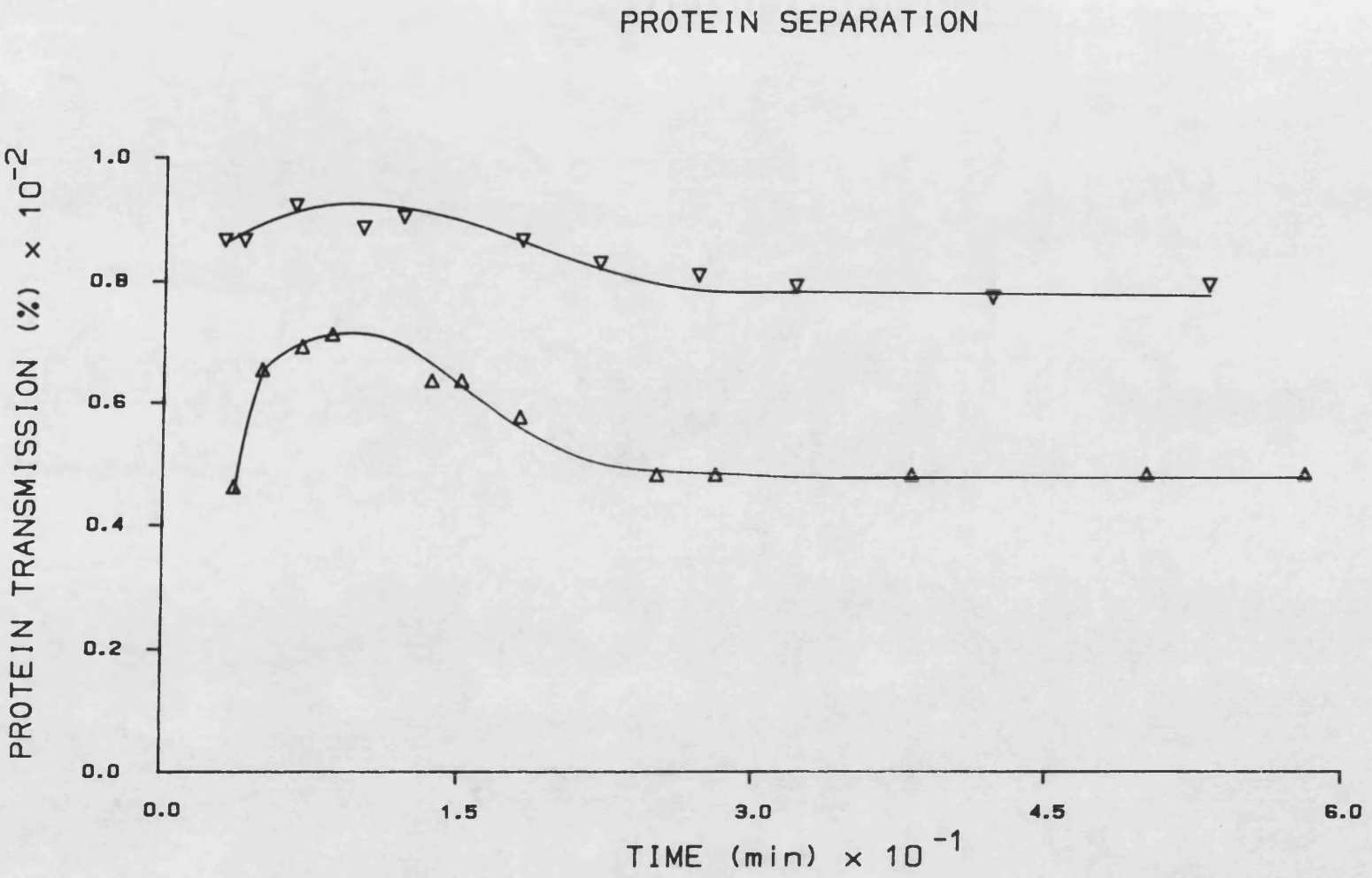


Fig.62: Feed: Bakers Yeast; 0.1 M Potassium Phosphate; pH=7.4; T=10°C; Pore size=0.45 μm; ΔP=14 kPa; Recycle Flow Rate=0.4 ℓ/min; Δ PTFE; ▽ CA;

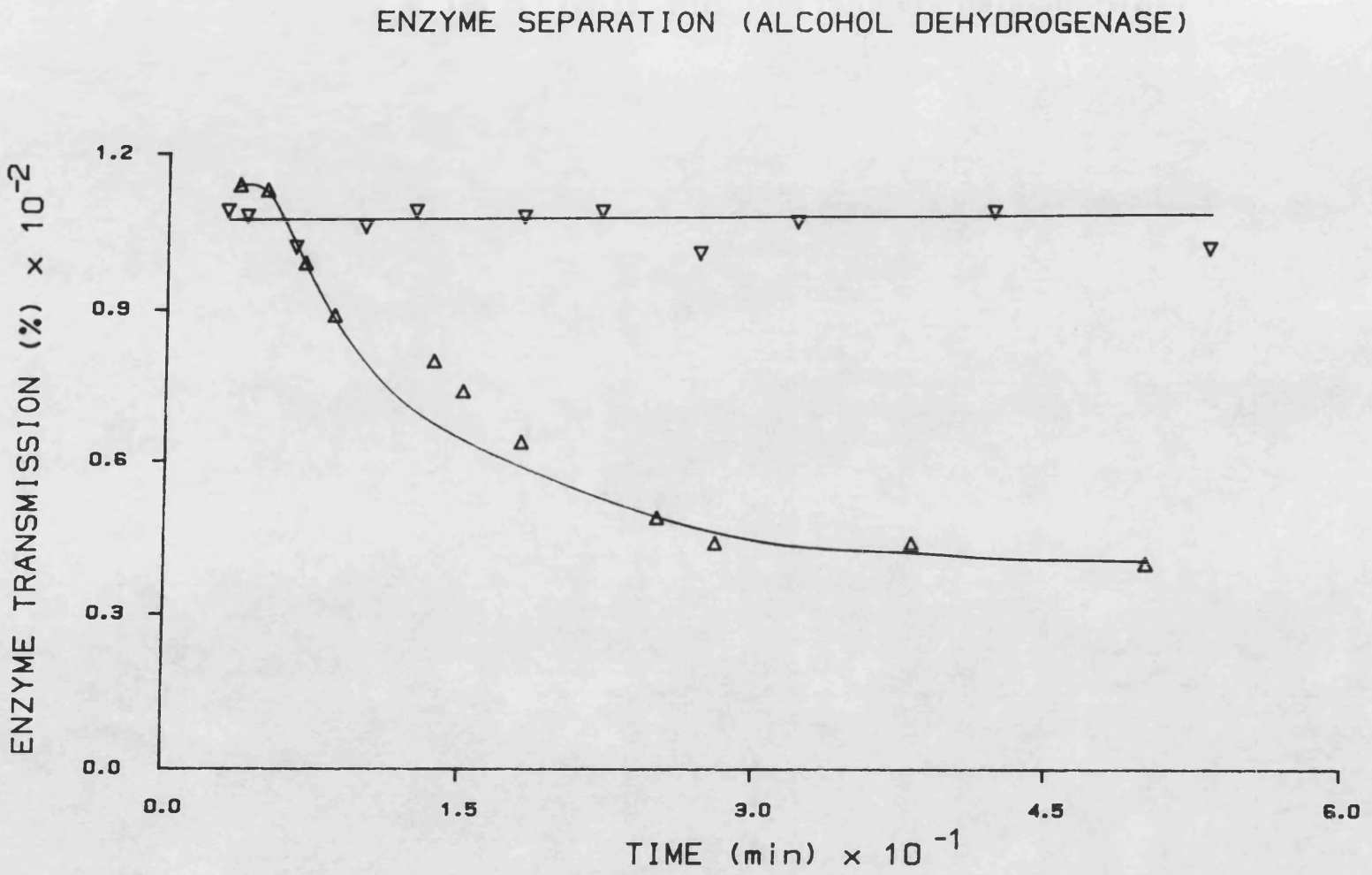


Fig.63: Feed: Bakers Yeast; 0.1 M Potassium Phosphate; pH-7.4; T-10°C; Pore size-0.45 μm; ΔP-14 kPa; Recycle Flow Rate-0.4 ℓ/min; Δ PTFE; ▽ CA;

permeability.

The incompatibility of hydrophobic PTFE membranes with proteins is clearly demonstrated in Figs.62 and 63.

The hydrophilic cellulose acetate membrane transmitted 83% of the protein whereas the PTFE membrane showed a 50% rejection. The difference is even more pronounced in the case of enzyme transmission. Cellulose acetate is non-rejecting whilst alcohol-dehydrogenase transmission by PTFE was only 44% with a tendency to decline further.

The fact that hydrophobic membranes are more rejecting than membranes which are hydrophilic in nature was attributed to enhanced protein adsorption due to hydrophobic membrane-protein interactions resulting in a higher free energy. In addition hydrophobic adsorption is irreversible in contrast to hydrophilic adsorption (Dillman and Miller, 1973).

This result indicates that cross-flow microfiltration is potentially applicable in yeast debris removal with some restriction on flux performance provided the membrane is of hydrophilic nature.

Part V

Cleaning

Membranes, once exposed to proteinaceous solutions, tend to change their original properties to transmit solutes and also show a tremendous decline in permeability with elapsing time.

In cases where protein separation is the main objective an increased rejection causes an undesired loss of product which might be recovered, at best partially by introducing an additional diafiltration step. Simultaneously a reduced flux equates with a longer processing time, hence increased running costs are incurred. Cleaning becomes necessary to recover the initial characteristics which sometimes is only achieved by applying harsh treatments. Industrial plants processing biologicals are subject to hygienic regulations and need to be sanitised frequently. Both cleaning and sanitising operations can be quite destructive for the membranes and it is generally thought that membrane lifetime is a function of the cleaning and sanitising it requires rather than the operating conditions which it undergoes. If cleaning is damaging the membrane its retention properties can deteriorate over time until replacement is the ony economically justifiable action.

Cleaning of membranes is an often neglected area of study and most of the data available is proprietary and in the hands of manufacturers. Part V describes the cleaning performance of some chemicals and biochemicals used in this work to restore the properties of CFP-polysulphone hollow fibre membranes.

Prior to cleaning the membranes were exposed to whey protein solutions (1.5g/ℓ) over a period of 60-80 min at 10°C.

The cleaning conditions are listed in Table 13. After completing the cleaning run the filtration unit was rinsed with water (55°C) for 5 min. In order to establish the cleaning efficiency the flux of water containing 0.1 M NaCℓ was measured at 10°C and compared to the steady state flux of a new membrane.

The results are listed in Table 13 and will be discussed separately.

1. Sodium Perborate ($NaBO_3 \times 4H_2O$)

Belfort (1974) reported that sodium perborate had been successfully used to give 80–85% flux restoration on cellulose acetate RO membranes but he also pointed out that a major drawback was the high pH (>9) at which cellulose acetate is likely to suffer from severe damage.

The membranes that were challenged with the cleaning chemicals were CFP–polysulphone membranes, able to withstand a range of pH 2–11 according to the manufacturer's recommendation. However, cleaning with sodium perborate recovered the original water flux by only 26%. Presumably perborate removes only loosely attached proteins by breaking down the molecules to sub–units through oxidation but does not completely detach tightly adsorbed proteins.

2. Hydrogen Peroxide

Le (1984) used hydrogen peroxide successfully as a cleaner except that it acted very slowly on foulants hence a long cleaning time was required. Hydrogen peroxide is also an oxidising agent and like sodium perborate restores the water flux by only 30%. presumably the same explanation given for sodium perborate applies to hydrogen peroxide.

3. Terg–A–Zyme

Biological detergents containing proteases to degrade the fouling layer are commonly used in industry.

Terg–A–Zyme is an alkaline enzyme active detergent with an optimum activity at a temperature of 50–55 °C. Proteases break down the proteins and the detergent effect removes the fragments from the surface once they have been rendered more water soluble with fewer points of attachment per molecule. Terg–A–Zyme restored 48% of the water flux.

4. Sodium Hydroxide

Like biological detergents sodium hydroxide is commonly in use as a cleaner. Its effect as a base decomposes proteins to sub-units and its detergent effect makes the fragments more water soluble.

Sodium hydroxide restored 50% of the original water flux.

5. Protamine Sulphate

Protamine sulphate is not a cleaner but a protein itself and its cleaning effect was most surprising. In restoring 54% of the original water flux it showed the best cleaning effect.

Protamine sulphate has a low molecular weight (~ 5000 MW) and is highly basic (I.E.P ~ 11). Its ready formation of salts and the tendency to associate with acidic proteins makes it react as a complexing agent comparable to EDTA which sequesters calcium.

The remaining complex is only loosely attached to the membrane and more easily removed.

TABLE 13 The Effect of Cleaning Chemicals on CFP Membranes

The initial steady state water flux of a new membrane was 900 ℓ/m²/h (10°C)

Water flux before cleaning (ℓ/m²/h)	Cleaning Chemical	Water flux after cleaning (ℓ/m²/h)	Relative Recovery of flux with respect to the steady state flux of a new membrane at 10°C
250 (55°C)	H_2O_2. 1g/ℓ, 55°C, 5 hrs	470, 55°C (270, 10°C)	30%
160 (55°C)	NaOH, 1g/ℓ, 55°C. 2 hrs	650, 55°C (450, 10°C)	50%
130 (55°C)	Terg-A-Zyme, 3 g/ℓ, 55°C, 2 hrs	620, 55°C (430, 10°C)	48%
10 (10°C)	$NaBO_3$*$4H_2O$, 1 g/ℓ, 55°C, 2 hrs	230, 10°C	26%
173 (10°C)	Protamine Sulphate, 2 g/ℓ, 10°C, 2 hrs	490, 10°C	54%

* All experiments were performed with the CFP-2D-3 membrane except cleaning with protamine sulphate which was performed with the CFP-2E-3 membrane.

The former results indicate that cleaning does not seem to restore the initial water flux of a new membrane, an observation which has been made by many others too, and it was also noticed that the flux declined progressively with extended use of the membrane. Presumably once the proteins have become adsorbed to the membrane they form very strong links which are decreasingly susceptible to be broken-down with proceeding consolidation of the fouling layer.

Sodium hydroxide and Terg-A-Zyme achieved the best cleaning effect. Sodium perborate and hydrogen peroxide were applied less successfully; besides some evidence was given that they caused damage to the membrane due to their extreme reactivity. It is therefore strongly recommended to use them with care.

Protamine sulphate gave the best cleaning performance and this at very mild conditions, which is important with respect to the membrane lifetime.An application as membrane cleaner on an industrial and also bench scale is definitely unrealistic with respect to costs. A relative cost comparison break down of sodium hydroxide, protamine sulfate and Terg-A-Zyme is given below:

Table 14

Cost of Cleaning Chemicals: Comparison between Sodium Hydroxide, Terg-A-Zyme and Protamine Sulfate (Calculated as a Percentage of the Cost of NaOH)

Chemical	Cost per cleaning run £	Relative Cost %
Sodium hydroxide	3.5×10^{-3}	100
Terg-A-Zyme	1.3×10^{-2}	370
Protamine Sulphate	5.25	150 000

Despite the fact that sodium hydroxide was cheaper and achieved with 50% a better flux recovery than Terg-A-Zyme (48%) it was decided to use Terg-A-Zyme as a regular cleaner because it was believed that the biological detergent is less harmful with respect to membrane life.

CHAPTER V

CONCLUSIONS

This work on the problems of fouling in cross-flow microfiltration led to the following conclusions:

(i) Flux and protein transmission are not necessarily determined by the same process and it is recommended that both cases should be considered separately:

Flux is determined by the increasing resistance exhibited by the fouling layer. This in turn will depend on the porosity as well as the thickness of the layer. The layer appears less permeable when salt is present (eg Fig.19) or when the proteins are at their I.E.P (eg Fig.17). The layer becomes more permeable when the proteins are charged as the pH moves away from the I.E.P (eg Fig.31).

Protein transmission is determined by protein adsorption to the membrane which is affected by the interactions of the charged protein with the diffuse double layer of the membrane. Transmission was found to be lowest at a pH where proteins are charged and highest when proteins are at their I.E.P (eg Fig.16 and Fig.27). Addition of salt (dipotassium hydrogen orthophosphate) is thought to compact the diffuse layer and thereby increase protein transmission (eg Fig.18).

(ii) The ratio of particle size to pore size affects flux, as illustrated during cell concentration (Fig.48). Membranes with a pore size of 0.45 μm gave the highest flux compared to membranes of 0.2 μm and 0.6 μm where particle deposition and pore plugging are likely to occur.

The ratio of particle size to pore size has also a major influence on protein transmission. This was demonstrated with the case of cell debris removal (eg Fig.58). Membranes with a pore size of 0.2 μm transmitted more proteins than

membranes of 0.2 μm and 0.6 μm which was ascribed to particle deposition and pore plugging.

(iii) Protein transmission increases with decreasing transmembrane pressure (Fig.41).

(iv) The flux of casein varied less over the range of pH compared to other proteins (comp. Fig.21 and Fig.23). This was attributed to the formation of colloids and their weaker response to changing charge conditions. Colloidal suspensions also showed a moderate minimum at the I.E.P whilst other proteins showed a maximum under these environmental conditions (comp Fig.20 and Fig.22). Hence, it was concluded that protein solutions and suspensions should be considered separately.

(v) Membranes change their rejection and flux properties once they have been exposed to proteinaceous material. The membrane permeability is then determined by the adsorbed fouling layer and its response to the ionic environment and pH (Fig.13).

(vi) Cross-flow microfiltation can concentrate fermentation broths; it also can recover and clarify intracellular enzymes.

(vii) Cleaning with NaOH, Terg-A-Zyme and Protamine sulphate restored only about 50% of the original water flux of a new membrane. Repeated use and cleaning with Terg-A-Zyme could maintain a reproducable flux thereafter.

(viii) In cases of protein solutions at the I.E.P and cell concentrations the apparent order n of the reaction process by which the fouling process was modelled was found to be between 1 and 2 when the fouling rate equation (equ. 9) was fitted to the data. The apparent order n varried at a pH away from the I.E.P.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE WORK

The work presented here has shown that changed charge conditions induced by pH and ionic concentration strongly affect membrane performance. To clarify the mechanisms occuring at the membrane surface the following set of experiments are recommended:

(i) Nassauer (1985) investigated the adsorption of proteins to membranes by means of zeta potential measurements. The method is described elsewhere (1984) and could be applied to establish the charge conditions at the membrane surface prior and after exposing it to protein solutions. This could help to define the extent of fouling under different environmental conditions and establish the membrane charge at which fouling is less likely. The next step would be to induce this charge to membranes by applying an electrical field.

The zeta potential measurement method is also suitable to investigate whether after cleaning non-removed foulants are primarily responsible for the reduced flux or if other influencing factors are evident.

(ii) The geometrical hindrance effect of the diffuse double layer on flux and transmission should be explored further. Membranes of defined pore sizes (eg Nuclepore, Anopore) could be exposed to pure water and solutions with defined ionic strengths of differently charged ions. Sodium, magnesium and ferric chloride could be used to investigate the relationship between increasing valency and compaction of the diffuse double layer. Another interesting question is whether anions have the same compressing effect as cations. Separately changing the valency of the positive and negativeions in added salts could provide this information.

Simultaneously it is suggested to measure the conductivities of the retentates and permeates. This could clarify if the double layer determines the flux and transmission behaviour or if there are ion exchange processes occuring at the membrane surface.

This work has shown that the pore to particle size ratio affects flux during cell concentration and also protein transmission, in particular during cell debris removal. To investigate this further the following set of experiments is recommended:

(iii) Establishing the favourable ratio would help to predict the optimum pore size. Membranes with defined pore sizes (eg Nuclepore, Anopore) could be used to filter particles of a defined size. It is suggested to use non-proteinaceous (eg dextran) particles to avoid or minimise adsorption effects. This could be extended to establish the impacts of adsorption processes and geometrical hindrance on flux and transmission by replacing the non- proteinaceous particles by microorganisms.

(iv) An interesting but puzzling result was a 110% enzyme transmission (Fig. 58) whilst the total protein transmission was less than 90%. This suggests that enzyme inhibiting proteins are retained and the specific enzyme activity in the permeate is therefore increased. Electrophoresis or HPLC could be used to determine the composition of the retentate and permeate.

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APPENDIX I

Listing of the computer program controlling air injection, timing the passage of the air bubble, recording the output of the UV and processing the data.

(BBC Basic)

```
  10 *KEY10OLD|ML.|M
  20 *FX 5,1
  30 *FX 229,1
  40 LOMEM=LOMEM+&800
  50 MODE7
  60 PORT=&FE60
  70 ?(PORT+2)=&FC:REM BITS 0,1 INPUTS
  80 ?PORT=128:REM PULSE OUTPUT HIGH
  90
 100
 110
 120 PRINT "DO YOU WANT THE MENU Y/N ? ":A$=GET$:REM TO LOAD ANY RESULT FROM
SC AT ANY TIME
 130 IF A$="Y" THEN GOTO 750:REM SENDS PROGRAM TO MENU
 140
 150
 160
 170 PRINT "ENTER STARTING CONDITIONS --- "
 180
 190
 200
 210 INPUT''"DATE (dd/mm/yy) ";DA$:$(TOP+4)=DA$
 220 INPUT'"Membrane ";MEM$:$(TOP+20)=MEM$
 230 INPUT'"AREA (SQRCM) ";AE$:$(TOP+36)=AE$:AREA=VAL(AE$)
 240 INPUT'"Pressure (PSI) ";PRESS$:$(TOP+44)=PRESS$
 250 INPUT'"Temperature (DEG. C) ";TEMP$:$(TOP+52)=TEMP$
 260 INPUT'"PH ";PH$:$(TOP+60)=PH$
 270 INPUT'"IONIC STRENGTH (MMOL/L) ";IO$:$(TOP+68)=IO$
 280 INPUT'"FEED ";FD$:$(TOP+76)=FD$
 290 INPUT'"DISTANCE (MM)";DI$:$(TOP+100)=DI$:DISTANCE=VAL(DI$)
 300 INPUT'"DILUTIONFACTOR";FA$:$(TOP+108)=FA$:DILUTION=VAL(FA$)
 310
 320
 330
 340 PROCON
 350 TIME=0:INDEX=TOP+120
 360 INPUT'"Pump running - Press RETURN to start ";A$
 370 PRINT'"Press Escape key for menu"
 380
 400
 410 PW=3:REM OPENING TIME FOR SOLENOID
 420 IF PW<10THEN PROCPULSE(PW) ELSE PROCERROR
 430 TW=TIME:REM TIME OF AIR FROM SOL.TO  PC1
 440 REPEAT:X=INKEY(-113):UNTIL (?PORT AND 1)=1 OR TIME-TW>3000 OR X=-1:REM SOL
ENOID OPENS UNTIL EITHER ESCAPE OR PC1 AC. OR NO BUBBLE INJECTED
 450 IF X=-1 THEN 750
 460 IF TIME-TW>3000 THEN PW=PW+1:GOTO 420
 470
 480
 490
 500 TW=TIME:REM TIME OF AIR FROM PC1 TO PC2
 510 REPEAT:UNTIL TIME-TW>12000 OR (?PORT AND 2)=2
 520 TF=TIME-TW:REMIF TF<50 THEN 740 ELSE IF TF>12000 THEN 400
 530
 540
 550
 560 A%=0
 570 FOR N%=1 TO 1000 STEP1
 580 A%=A%+ADVAL(1)DIV32
 590 NEXT
 600 UV%=A%/1000:PROCSTORE
 610
 620
 630
```

```
  640 TW=TIME
  650 IF TW<180000 THEN 660 ELSE 690
  660 TW=TIME
  670 REPEAT:X=INKEY(-113):UNTIL TIME-TW>6000 OR X=-1
  680 IF X=-1 THEN 750 ELSE 410
  690 TW=TIME
  700 REPEAT:X=INKEY(-113):UNTIL TIME-TW>30000 OR X=-1
  710 IF X=-1 THEN 750 ELSE 410
  720
  730
  740
  750 PROCMENU
  760 A$=GET$
  770 ON INSTR("123456",A$) GOTO 780,790,810,870,880,890 ELSE 760
  780 CLS:PROCHEADER:GOTO 370
  790 PROCPRINTER:CLS:PRINT "START PUMP Y/N ":A$=GET$:IF A$="Y" THEN GOTO 170
  800 GOTO 750
  810 CLS:PRINT" DO YOU WANT PROCSCALEFAST OR PROCSCALESLOW,FAST=F,SLOW=S,MEDIUM
=M":REPEAT:A$=GET$:UNTIL A$="F" OR A$="S"OR A$="M":MODE 4
  820 IF A$="F" THEN PROCSCALEFAST:PROCPLOTFAST:MODE7:GOTO 750
  830 IF A$="S" THEN PROCSCALESLOW:PROCPLOTSLOW:MODE7:GOTO 750
  840 IF A$="M" THEN PROCSCALEMED:PROCPLOTMED:MODE7:GOTO 750
  850
  860
  870 PROCDISC:GOTO 750
  880 PROCLOAD:GOTO 750
  890 CLS:PRINT" DO YOU WANT PROCSCHIGH OR PROCSCLOW,HIGH=H,LOW=L":REPEAT:B$=GET
$:UNTIL B$="H" OR B$="L":MODE 4
  900 IF B$="H" THEN PROCSCHIGH:PROCCHIGH:MODE7:GOTO 750
  910 IF B$="L" THEN PROCSCLOW:PROCCLOW:MODE7:GOTO 750
  920
  930
  940
  950 DEF PROCPULSE(CS)
  960 X=?PORT
  970 ?PORT=X AND 127
  980 TP=TIME
  990 REPEAT:UNTIL TIME-TP>CS
 1000 ?PORT=X OR 128
 1010 ENDPROC
 1040
 1050 DEF PROCON
 1060 X=?PORT
 1070 ?PORT=X OR 64
 1080 ENDPROC
 1090
 1100
 1110
 1120 DEF PROCOFF
 1130 X=?PORT
 1140 ?PORT=X AND 191
 1150 ENDPROC
 1160
 1170
 1180
 1190 DEF PROCERROR
 1200 PRINT"NO PULSES BEING RECEIVED"
 1210 X=GET
 1220 ENDPROC
 1230
 1240
 1250
```

```
 1260 DEF PROCSTORE
 1270 !INDEX=TIME
 1280 INDEX!4=TF
 1290 INDEX!6=UV%
 1300 IF INDEX-TOP<&7F8THEN INDEX=INDEX+8 ELSE PRINTTAB(1,12);"OUT OF MEMORY":EN
DPROC
 1310 IF INDEX-TOP>&760THEN PRINTTAB(1,12);"MEMORY??????"
 1320 ENDPROC
 1330
 1340
 1350
 1360 DEF PROCMENU
 1370 VDU 22,7
 1380 PRINTTAB(1,1);"(1) CONTINUE EXPERIMENT"
 1390 PRINTTAB(1,3);"(2) SEND RESULTS TO PRINTER"
 1400 PRINTTAB(1,5);"(3) PLOT FLUX VS TIME ON SCREEN"
 1410 PRINTTAB(1,7);"(4) SAVE RESULTS ON DISC"
 1420 PRINTTAB(1,9);"(5) LOAD RESULTS FROM DISC"
 1430 PRINTTAB(1,11);"(6) PLOT CONC VS TIME ON SCREEN"
 1440 ENDPROC
 1450
 1460
 1470
 1480 DEF PROCPRINTER
 1490 CLS
 1500 PRINT"Do you want a printed record Y/N"'':A$=GET$
 1510 IF A$="Y" THEN VDU2
 1520 CLS
 1530 PROCHEADER:PRINT'
 1540 FOR N=TOP+120 TO INDEX-1 STEP 8
 1550 TT=?(N+4)+256*?(N+5)
 1560 PRINT TT
 1570 REM IF TT=0 THEN  PRINT'"FLUX= ";0:GOTO 1550
 1580 C=?(N+6)+256*?(N+7)-187
 1590 PRINT C
 1600 PRINT'" At Time(MIN)= ";!N/100/60;"FLUX(L/H/M2)= ";13.2*DISTANCE*3600/(?(N
+4)+256*?(N+5))/AREA;:IF C<0 THEN PRINT' "CONC(G/L)= ";0 ELSE PRINT' "CONC(G/L)=
 ";(?(N+6)+256*?(N+7)-187)*0.01353*DILUTION
 1610 NEXT
 1620 REM 10 INCLUDES THE CROSSECTIONAL AERA PLUS CORRECTION PLUS THE CONVERTION
FACTOR OF UNITS FLUX IS IN L/H/M2
 1630 REM 187 IS THE BASELINE AND HAS TO BE SUBTRACTED .01353REPRESENTS THE SLOP
 1640 VDU3
 1650 X$=GET$
 1660 ENDPROC
 1670
 1680
 1690
 1700
 1710 DEF PROCPLOTSLOW
 1720 XMIN=(!(TOP+120))/100/60
 1730 MOVE 200+XMIN/150*750,100+13.2*DISTANCE*3600*4/(?(TOP+124)+256*?(TOP+125
/AREA
 1740 FOR N=TOP+128 TO INDEX-1 STEP 8
 1750 X=200+((!N)/100/60)/150*750
 1760 Y=100+13.2*DISTANCE*3600*4/(?(N+4)+256*?(N+5))/AREA
 1770 DRAW X,Y
 1780 NEXT
 1790 A$=GET$:IF A$="Y" THEN *GDE
 1800 X$=GET$
 1810 ENDPROC
 1820
 1830
 1840
```

```
 1850 DEF PROCPLOTFAST
 1860 XMIN=(!(TOP+120))/100/60
 1870 MOVE 200+XMIN/150*750,100+13.2*DISTANCE*3600*0.5/(?(TOP+124)+256*?(TOP+1:
))/AREA
 1880 FOR N=TOP+128 TO INDEX-1 STEP 8
 1890 X=200+((!N)/100/60)/150*750
 1900 Y=100+0.5*DISTANCE*3600*13.2/(?(N+4)+256*?(N+5))/AREA
 1910 DRAW X,Y
 1920 NEXT
 1930 A$=GET$:IF A$="Y" THEN *GDE
 1940 X$=GET$
 1950 ENDPROC
 1960
 1970
 1980 DEF PROCPLOTMED
 1990 XMIN=(!(TOP+120))/100/60
 2000 MOVE 200+XMIN/150*750,100+13.2*DISTANCE*3600*0.8/(?(TOP+124)+256*?(TOP+1:
))/AREA
 2010 FOR N=TOP+128 TO INDEX-1 STEP 8
 2020 X=200+((!N)/100/60)/150*750
 2030 Y=100+0.8*DISTANCE*3600*13.2/(?(N+4)+256*?(N+5))/AREA
 2040 DRAW X,Y
 2050 NEXT
 2060 A$=GET$:IF A$="Y" THEN *GDE
 2070 X$=GET$
 2080 ENDPROC
 2090
 2100
 2110 DEF PROCDISC
 2120 INPUT"NAME OF FILE ";NAME$
 2130 !TOP=INDEX
 2140 CLS
 2150 PRINT"SAVE ";NAME$;" ";~TOP;" +800 "
 2160 ?&7C1F=13
 2170 X%=0
 2180 Y%=&7C
 2190 CALL &FFF7
 2200 ENDPROC
 2210
 2220
 2230
 2240 DEF PROCLOAD
 2250 INPUT"NAME OF FILE ";NAME$
 2260 CLS
 2270 PRINT"*LOAD ";NAME$;" ";~TOP
 2280 ?&7C1F=13
 2290 X%=0
 2300 Y%=&7C
 2310 CALL &FFF7
 2320 INDEX=!TOP
 2330 DA$=$(TOP+4):MEM$=$(TOP+20):AE$=$(TOP+36):AREA=VAL(AE$):PRESS$=$(TOP+44):T
EMP$=$(TOP+52):PH$=$(TOP+60):IO$=$(TOP+68):FD$=$(TOP+76):DI$=$(TOP+100):DISTANCE
=VAL(DI$):FA$=$(TOP+108):DILUTION=VAL(FA$)
 2340 ENDPROC
 2350
 2360
 2370
```

```
2380 DEF PROCHEADER
2390 PRINT"DATE=";DA$
2400 PRINT"MEMBRANE=";MEM$
2410 PRINT"AREA= ";AE$;" SQRCM"
2420 PRINT"Pressure= ";PRESS$;" Bar"
2430 PRINT"Temperature= "TEMP$;" DEG. C"
2440 PRINT"pH= ";PH$
2450 PRINT"IONIC STRENGTH= ";IO$;" MMOL/L"
2460 PRINT"FEED= ";FD$
2470 PRINT"DISTANCE= ";DI$;" MM"
2480 PRINT"DILUTIONFACTOR= ";FA$
2490 ENDPROC
2500
2510
2520
2530
2540 DEF PROCSCALESLOW
2550 CLS
2560 MOVE 200,100
2570 DRAW 950,100
2580 DRAW 950,900
2590 DRAW 200,900
2600 DRAW 200,100
2610 MOVE 300,100:DRAW 300,110
2620 MOVE 400,100:DRAW 400,110
2630 MOVE 500,100:DRAW 500,110
2640 MOVE 600,100:DRAW 600,110
2650 MOVE 700,100:DRAW 700,110
2660 MOVE 800,100:DRAW 800,110
2670 MOVE 900,100:DRAW 900,110
2680 MOVE 200,200:DRAW 210,200
2690 MOVE 200,300:DRAW 210,300
2700 MOVE 200,400:DRAW 210,400
2710 MOVE 200,500:DRAW 210,500
2720 MOVE 200,600:DRAW 210,600
2730 MOVE 200,700:DRAW 210,700
2740 MOVE 200,800:DRAW 210,800
2750 PRINTTAB(4,28);"0"
2760 PRINTTAB(3,25);"25"
2770 PRINTTAB(3,22);"50"
2780 PRINTTAB(3,19);"75"
2790 PRINTTAB(2,16);"100"
2800 PRINTTAB(2,13);"125"
2810 PRINTTAB(2,10);"150"
2820 PRINTTAB(2,7);"175"
2830 PRINTTAB(0,3);"FLUX"
2840 PRINTTAB(0,4);"L/H/M2"
2850 PRINTTAB(6,29);"0"
2860 PRINTTAB(9,29);"20"
2870 PRINTTAB(12,29);"40"
2880 PRINTTAB(15,29);"60"
2890 PRINTTAB(18,29);"80"
2900 PRINTTAB(21,29);"100"
2910 PRINTTAB(24,29);"120"
2920 PRINTTAB(27,29);"140"
2930 PRINTTAB(26,30);"TIME"
2940 PRINTTAB(26,31)"MIN";
2950 ENDPROC
2960
2970
```

```
2980 DEF PROCSCALEFAST
2990 CLS
3000 MOVE 200,100
3010 DRAW 950,100
3020 DRAW 950,900
3030 DRAW 200,900
3040 DRAW 200,100
3050 MOVE 300,100:DRAW 300,110
3060 MOVE 400,100:DRAW 400,110
3070 MOVE 500,100:DRAW 500,110
3080 MOVE 600,100:DRAW 600,110
3090 MOVE 700,100:DRAW 700,110
3100 MOVE 800,100:DRAW 800,110
3110 MOVE 900,100:DRAW 900,110
3120 MOVE 200,200:DRAW 210,200
3130 MOVE 200,300:DRAW 210,300
3140 MOVE 200,400:DRAW 210,400
3150 MOVE 200,500:DRAW 210,500
3160 MOVE 200,600:DRAW 210,600
3170 MOVE 200,700:DRAW 210,700
3180 MOVE 200,800:DRAW 210,800
3190 PRINTTAB(4,28);"0"
3200 PRINTTAB(2,25);"200"
3210 PRINTTAB(2,22);"400"
3220 PRINTTAB(2,19);"600"
3230 PRINTTAB(2,16);"800"
3240 PRINTTAB(1,13);"1000"
3250 PRINTTAB(1,10);"1200"
3260 PRINTTAB(1,7);"1400"
3270 PRINTTAB(0,3);"FLUX"
3280 PRINTTAB(0,4);"L/H/M2"
3290 PRINTTAB(6,29);"0"
3300 PRINTTAB(9,29);"20"
3310 PRINTTAB(12,29);"40"
3320 PRINTTAB(15,29);"60"
3330 PRINTTAB(18,29);"80"
3340 PRINTTAB(21,29);"100"
3350 PRINTTAB(24,29);"120"
3360 PRINTTAB(27,29);"140"
3370 PRINTTAB(26,30);"TIME"
3380 PRINTTAB(26,31)"MIN";
3390 ENDPROC
3400
3410
3420 DEF PROCSCHIGH
3430 CLS
3440 MOVE 200,100
3450 DRAW 950,100
3460 DRAW 950,900
3470 DRAW 200,900
3480 DRAW 200,100
3490 MOVE 300,100:DRAW 300,110
3500 MOVE 400,100:DRAW 400,110
3510 MOVE 500,100:DRAW 500,110
3520 MOVE 600,100:DRAW 600,110
3530 MOVE 700,100:DRAW 700,110
3540 MOVE 800,100:DRAW 800,110
3550 MOVE 200,200:DRAW 210,200
```

```
3570 MOVE 200,400:DRAW 210,400
3580 MOVE 200,500:DRAW 210,500
3590 MOVE 200,600:DRAW 210,600
3600 MOVE 200,700:DRAW 210,700
3610 MOVE 200,800:DRAW 210,800
3620 PRINTTAB(2,28);"0"
3630 PRINTTAB(2,25);"2.5"
3640 PRINTTAB(2,22);"5"
3650 PRINTTAB(2,19);"7.5"
3660 PRINTTAB(1,16);"10"
3670 PRINTTAB(1,13);"12.5"
3680 PRINTTAB(1,10);"15"
3690 PRINTTAB(1,7);"17.5"
3700 PRINTTAB(0,3);"CONC"
3710 PRINTTAB(0,4);"G/L"
3720 PRINTTAB(6,29);"0"
3730 PRINTTAB(9,29);"20"
3740 PRINTTAB(12,29);"40"
3750 PRINTTAB(15,29);"60"
3760 PRINTTAB(18,29);"80"
3770 PRINTTAB(21,29);"100"
3780 PRINTTAB(24,29);"120"
3790 PRINTTAB(27,29);"140"
3800 PRINTTAB(26,30);"TIME"
3810 PRINTTAB(26,31)"MIN";
3820 ENDPROC
3830,
3840
3850 DEF PROCCHIGH
3860 XMIN=(!(TOP+120))/100/60
3870 MOVE 200+XMIN/150*750,100+(?(TOP+126)+256*?(TOP+127)-187)*0.01353*DILUTION
*40.0
3880 FOR N=TOP+128 TO INDEX-1 STEP 8
3890 X=200+((!N)/100/60)/150*750
3900 C=?(N+6)+256*?(N+7)-187
3910 IF C<0THEN Y=100 ELSE Y=100+(?(N+6)+256*?(N+7)-187)*DILUTION*0.01353*40.0
3920 DRAW X,Y
3930 NEXT
3940 A$=GET$:IF A$="Y" THEN *GDE
3950 X$=GET$
3960 ENDPROC
3970 DEF PROCCLOW
3980 XMIN=(!(TOP+120))/100/60
3990 MOVE 200+XMIN/150*750,100+(?(TOP+126)+256*?(TOP+127)-187)*0.01353*DILUTION
*200
4000 FOR N=TOP+128 TO INDEX-1 STEP 8
4010 X=200+((!N)/100/60)/150*750
4020 C=?(N+6)+256*?(N+7)-187
4030 IF C<0THEN Y=100 ELSE Y=100+(?(N+6)+256*?(N+7)-187)*DILUTION*0.01353*200
4040 DRAW X,Y
4050 NEXT
4060 A$=GET$:IF A$="Y" THEN *GDE
4070 X$=GET$
4080 ENDPROC
4090
4100 DEF PROCSCALEMED
4110 CLS
4120 MOVE 200,100
4130 DRAW 950,100
4140 DRAW 950,900
4150 DRAW 200,900
4160 DRAW 200,100
4170 MOVE 300,100:DRAW 300,110
4180 MOVE 400,100:DRAW 400,110
4190 MOVE 500,100:DRAW 500,110
```

```
4210 MOVE 700,100:DRAW 700,110
4220 MOVE 800,100:DRAW 800,110
4230 MOVE 900,100:DRAW 900,110
4240 MOVE 200,200:DRAW 210,200
4250 MOVE 200,300:DRAW 210,300
4260 MOVE 200,400:DRAW 210,400
4270 MOVE 200,500:DRAW 210,500
4280 MOVE 200,600:DRAW 210,600
4290 MOVE 200,700:DRAW 210,700
4300 MOVE 200,800:DRAW 210,800
4310 PRINTTAB(4,28);"0"
4320 PRINTTAB(2,25);"125"
4330 PRINTTAB(2,22);"250"
4340 PRINTTAB(2,19);"375"
4350 PRINTTAB(2,16);"500"
4360 PRINTTAB(2,13);"625"
4370 PRINTTAB(2,10);"750"
4380 PRINTTAB(2,7);"875"
4390 PRINTTAB(0,3);"FLUX"
4400 PRINTTAB(0,4);"L/H/M2"
4410 PRINTTAB(6,29);"0"
4420 PRINTTAB(9,29);"20"
4430 PRINTTAB(12,29);"40"
4440 PRINTTAB(15,29);"60"
4450 PRINTTAB(18,29);"80"
4460 PRINTTAB(21,29);"100"
4470 PRINTTAB(24,29);"120"
4480 PRINTTAB(27,29);"140"
4490 PRINTTAB(26,30);"TIME"
4500 PRINTTAB(26,31)"MIN";
4510 ENDPROC
4520 DEF PROCSCLOW
4530 CLS
4540 MOVE 200,100
4550 DRAW 950,100
4560 DRAW 950,900
4565 DRAW 200,900
4570 DRAW 200,100
4580 MOVE 300,100:DRAW 300,110
4590 MOVE 400,100:DRAW 400,110
4600 MOVE 500,100:DRAW 500,110
4610 MOVE 600,100:DRAW 600,110
4620 MOVE 700,100:DRAW 700,110
4630 MOVE 800,100:DRAW 800,110
4640 MOVE 200,200:DRAW 210,200
4650 MOVE 200,300:DRAW 210,300
4660 MOVE 200,400:DRAW 210,400
4670 MOVE 200,500:DRAW 210,500
4680 MOVE 200,600:DRAW 210,600
4690 MOVE 200,700:DRAW 210,700
4700 MOVE 200,800:DRAW 210,800
4710 MOVE 200,700:DRAW 210,700
4720 MOVE 200,800:DRAW 210,800
4730 PRINTTAB(2,28);"0"
4740 PRINTTAB(2,25);"0.5"
4750 PRINTTAB(2,22);"1"
4760 PRINTTAB(2,19);"1.5"
4770 PRINTTAB(1,16);"2"
4780 PRINTTAB(1,13);"2.5"
4790 PRINTTAB(1,10);"3"
4800 PRINTTAB(1,7);"3.5"
4810 PRINTTAB(0,3);"CONC"
4820 PRINTTAB(0,4);"G/L"
4830 PRINTTAB(6,29);"0"
4840 PRINTTAB(9,29);"20"
```

```
4860 PRINTTAB(15,27); 80
4870 PRINTTAB(18,29);"80"
4880 PRINTTAB(21,29);"100"
4890 PRINTTAB(24,29);"120"
4900 PRINTTAB(27,29);"140"
4910 PRINTTAB(26,30);"TIME"
4920 PRINTTAB(26,31)"MIN";
4930 ENDPROC
```

Listing of the computer program optimising k_R and n using a Simplex routine and a Fast-Butcher integration routine

(Microsoft Basic)

```
1000 REM ************** DYFIT ******************
1010 REM
1030 REM
1040 REM Program fits parameters in a differential equation
1050 REM using a simplex routine.
1060 REM
1070 REM Original simplex routine taken from BYTE May 1984
1080 REM
1090 REM Numerical integration uses the Fast-Butcher routine
1100 REM as written at U.C. Swansea
1110 REM
1120 CLS
1130 DIM PL(50),PH(50):PM=10
1140 DIM RR(1):ZZ=1
1150 DIM W(15),YO(5),Y(5),D(5),W1(5),W2(5)
1160 DIM W3(5),W4(5),W5(5),Y1(5)
1170 DIM MAT(20,20),A(20),SUM(20),RI(20)
1180 DIM Y9(5),YR(100)
1190 DEFINT I,J,H,L
1200 M=2                                        :REM No paramters
1210 NB=M+1 : A$="££££.£££££"
1220 DIM SIMP(20,3),ST(100),MA(100),D1(100),D2(100)
1230 DIM XX(50),X(50),P(50),Q(50),L(50),H(50)
1240 DIM CENTRE(50),NXT(50),ER(50),MEAN(50)
1250 GOSUB 3960: REM  GOTO PARAMETR INPUT SUBROUTINE
1260 ROOT2 = 1.414214
1270 ALPHA = 1                                 :REM Reflection coefficient
1280 BETA  = .5                                 :REM Contraction coefficient
1290 GAMMA = 2                                 :REM Expansion coefficient
1300 TR = 1 : FAL = 0
1310 REM FUNCTION F combined in SSR routine
1320 GOTO 1830                                   :REM Jump to start of program
1330 REM ------------------------------------------------------------------
1340 REM SUBROUTINE SSR
1350 X(NB)=0
1360 GOSUB 3500: REM  GOTO NUMERICAL INTEGRATION ROUTINE
1370 FOR I1 = 1 TO NP
1380   X(NB) = X(NB) +((YR(I1) - D2(I1)) * (YR(I1) - D2(I1)))
1390 NEXT I1
1400 RETURN
1410 REM ------------------------------------------------------------------
1420 REM Subroutine ORDER
1430 FOR J = 1 TO NB
1440   FOR I = 1 TO NB
1450     IF SIMP(I,J) < SIMP(L(J),J) THEN L(J) = I
1460     IF SIMP(I,J) > SIMP(H(J),J) THEN H(J) = I
1470   NEXT I
1480 NEXT J
1490 RETURN
1500 REM ------------------------------------------------------------------
1510 REM Subroutine NEW VERTEX
1520 NITER = NITER + 1 : CD = 559
1530 PRINT NITER
1540 FOR I = 1 TO NB
1550   CD = CD + 15
1560   SIMP(H(NB),I) = NXT(I)
1565   PRINT XX(1)
1566   PRINT XX(2)
1570   PRINT"***"
1580 NEXT I
1590 RETURN
1600 REM ------------------------------------------------------------------
```

```
1610 REM Subroutine REPORT
1620 PRINT
1630 PRINT"The program exited after ";NITER ;"iterations"
1640 PRINT"The final simplex is "
1650 FOR J = 1 TO NB
1660   FOR I = 1 TO NB
1670     PRINT SIMP(J,I),
1680   NEXT I
1690   PRINT
1700 NEXT J
1710 PRINT"The mean is "
1720 FOR I = 1 TO NB
1730   PRINT MEAN(I);
1740 NEXT I
1750 PRINT : PRINT"The estimated fractional error is :"
1760 FOR I = 1 TO NB
1770   PRINT ER(I),
1780 NEXT I
1790 PRINT:INPUT"Do you want to print out data";PLO$
1800 IF PLO$="NB" THEN GOTO 1820
1810 GOSUB 3360: REM PRINT OUT DATA
1820 END
1830 GOSUB 3050                              :REM Dummy read for testing
1840 XX(1) = SIMP(1,1) : XX(2) = SIMP(1,2)
1850 GOSUB 1350                             :REM Detm SSR for starting estimat
e
1860 SIMP(1,NB)=X(NB)
1870 REM
1880 FOR I = 1  TO M
1890   P(I) = ST(I) * (SQR(NB) + M - 1) / (M * ROOT2)
1900   Q(I) = ST(I) * (SQR(NB) - 1) / (M * ROOT2)
1910 NEXT
1920 REM
1930 FOR I = 2 TO NB
1940   FOR J = 1 TO M
1950     SIMP(I,J) = SIMP(1,J) + Q(J)
1960   NEXT J
1970   SIMP(I,I-1) = SIMP(1,I-1) + P(I-1)
1980   XX(1) = SIMP(I,1) : XX(2) = SIMP(I,2)
1990   GOSUB 1350                           :REM Detm SSR for other vertexes
2000   SIMP(I,NB)=X(NB)
2010 NEXT I
2020 REM
2030 REM Preset indicators for order subroutine
2040 FOR I = 1 TO NB
2050   L(I) = 1
2060   H(I) = 1
2070 NEXT I
2080 REM
2090 GOSUB 1430                              :REM Order vertexes
2100 REM
2110 REM Output starting simplex to the screen
2120 CLS
2130 PRINT"Starting Simplex"
2140 FOR J = 1 TO NB
2150   PRINT "Simp°";J;"$  ";
2160   FOR I = 1 TO NB
2170     PRINT SIMP(J,I),
2180   NEXT I
2190   PRINT
2200 NEXT J
2210 PRINT"                  * ITERATING *"
2220 PRINT:PRINT "    NB          Vm          Ke          SSR"
2230 REM
2240 NITER = 0                               :REM Initialise No. iterations
```

```
2250 REM

2260 REM Program will loop back to this point until done
2270 DUN = TR
2280 REM
2290 FOR I = 1 TO NB
2300   CENTRE(I) = 0
2310 NEXT I
2320 REM
2330 FOR I = 1 TO NB                              :REM Compute centroid
2340   IF I <> H(NB) THEN FOR J = 1 TO M:CENTRE(J) = CENTRE(J) + SIMP(I,J) :
         NEXT J
2350 NEXT I
2360 REM
2370 FOR I = 1 TO NB                              :REM 1st attempt to reflect
2380   CENTRE(I) = CENTRE(I) / M
2390   NXT(I) = (1 + ALPHA) * CENTRE(I) - ALPHA * SIMP(H(NB),I)
2400 NEXT I
2410 XX(1) = NXT(1) : XX(2) = NXT(2)              :REM Could be done inside loop
2420 GOSUB 1350                                    :REM Compute SSR
2430 NXT(NB) = X(NB)
2440 REM
2450 REM Check to see if change was beneficial or not
2460 IF NXT(NB) <= SIMP(L(NB),NB) THEN GOSUB 2700 ELSE GOSUB 2820
2470 REM
2480 REM Adjust order of vertexes
2490 GOSUB 1430
2500 REM Check for convergence
2510 FOR J = 1 TO NB
2520   ER(J) = (SIMP(H(J),J) - SIMP(L(J),J)) / SIMP(H(J),J)
2530   IF DUN =TR THEN IF ER(J) > MA(J) THEN DUN = FAL
2540 NEXT J
2550 IF DUN = FAL THEN IF NITER <= MXITER GOTO 2270
2560 REM
2570 REM Its converged or reached iteration limit
2580 REM Average each parameter
2590 FOR I = 1 TO NB
2600   MEAN(I) = 0
2610   FOR J = 1 TO NB
2620     MEAN(I) = MEAN(I) + SIMP(J,I)
2630   NEXT J
2640   MEAN(I) = MEAN(I) / NB
2650 NEXT I
2660 REM
2670 GOSUB 1620
2680 END
2690 REM Its <= the lowest simplex - thats very good
2700 GOSUB 1520                                    :REM Replace worst vertex
2710 FOR I = 1 TO M
2720   NXT(I) = GAMMA * SIMP(H(NB),I) + (1 - GAMMA) * CENTRE(I)
2730   XX(I) = NXT(I)
2740 NEXT I
2750 GOSUB 1350                                    :REM Compute SSR
2760 NXT(NB) = X(NB)
2770 REM If expansion is beneficial replace vertex
2780 IF NXT(NB) <= SIMP(L(NB),NB) THEN GOSUB 1520
2790 RETURN
2800 REM
2810 REM Its bigger than the lowest vertex but is it smaller than the highes
t
2820 IF NXT(NB) <= SIMP(H(NB),NB) THEN GOSUB 1520 : RETURN
2830 REM Lets try a contraction then
2840 FOR I = 1 TO M
2850   NXT(I) = BETA * SIMP(H(NB),I) + (1 - BETA) * CENTRE(I)
2860   XX(I) = NXT(I)
2870 NEXT I
```

```
2880 GOSUB 1350                                    :REM Compute SSR
2890 NXT(NB)= X(NB)

2900 REM
2910 REM Is the contraction acceptable
2920 IF NXT(NB) <= SIMP(H(NB),NB) THEN GOSUB 1520 : RETURN
2930 REM
2940 REM Its still bad then shrink bad vertexes
2950 FOR I = 1 TO NB
2960   FOR J = 1 TO M
2970     SIMP(I,J) = (SIMP(I,J) + SIMP(L(NB),J)) * BETA
2980     XX(J) = SIMP(I,J)
2990   NEXT J
3000   GOSUB 1350                                  :REM Compute SSR
3010   SIMP(I,NB) = X(NB)
3020 NEXT I
3030 RETURN
3040 REM
3050 REM READ IN DATA FOR SIMPLEX ROUTINE
3060 REM
3070 INPUT"Max number of iterations";MXITER
3080 INPUT"NUMBER OF PARAMETERS";M
3090 PRINT"ENTER INITIAL ESTIMATES OF THE PARAMETERS"
3100 FOR I=1 TO M
3110   INPUT SIMP(1,I)
3120 NEXT I
3130 PRINT"STEP SIZES ( <= 10% PARAMETER VALUE)"
3140 FOR I=1 TO M
3150   INPUT ST(I)
3160 NEXT I
3170 PRINT"MAXIMUM PERMITTED ERROR ON EACH PARAMETER"
3180 FOR I=1 TO M+1
3190   INPUT MA(I)
3200 NEXT I
3210 REM
3220 REM READ EXPERIMENTAL DATA FROM A DISK FILE
3230 REM
3240 INPUT"NAME OF DATA FILE";DAT$
3250 INPUT"DRIVE LETTER";DRI$
3260 DAT$=DRI$+":"+DAT$
3270 D=OPENIN DAT$
3280 INPUT£D,NP
3290 FOR I = 1 TO NP
3300   INPUT£D,D1(I),D2(I)
3310   PRINT I;D1(I),D2(I)
3320 NEXT I
3330 CLOSE £D
3340 RETURN
3350 REM
3360 REM DATA OUTPUT ROUTINE
3370 REM
3380 VDU2
3390 FOR I=1 TO NP
3400   PRINT D1(I),TAB(5),D2(I),TAB(5),YR(I),TAB(5),D2(I)-YR(I)
3410   ES=ES+(D2(I)-YR(I))^2
3420 NEXT I
3430 INPUT"DATA FILE NAME";F$
3440 F$="B:"+F$
3450 PRINT:PRINT
3460 PRINT"ERROR SUM =";ES
3470 VDU3
3480 RETURN
3490 REM
3500 REM DYMAMMIC SUBROUTINE
3510 REM
3520 YO(1)=YINIT:Y(1)=YINIT
```

```
3530 MMM=D1(1)
3540 REM X=TIME  Z=INCREMENTAL TIME INTERVAL  H=INTEGRATION INTERVAL
3550 X=0:W3=0

3560 N=1:JJ=1
3570 H=Z/2
3580 E9=1E-4
3590 GOSUB3680
3600 IF X<MMM THEN 3590
3610 YR(JJ)=Y0(1)
3620 PRINT X,YR(JJ)
3630 JJ=JJ+1:MMM=MMM+(D1(JJ)-D1(JJ-1)):IF MMM=0 THEN RETURN
3640 GOTO3590
3650 REM THE DIFFERENTIAL EQUATIONS
3660 D(1)=-(Y0(1)^2*EXP((XX(1)*Y0(1))/KM)*((XX(2)*U*CB^XX(1))/DPZ))
3670 RETURN
3680 K9=X+Z:K0=0:K8=X:FORK=1TON:Y9(K)=Y(K):NEXT:HH=0:IFW3=.375THEN3740
3690 REM N=3:DIM Y(N),Y0(N),Y1(N),Y9(2*N),D(N),W1(N),W2(N),W3(N),W4(N),W5(N)
3700 IFH>Z/2ORH<=0THENPRINT"INITIAL H="H"!";:H=Z/2:PRINT"DEFAULT="H
3710 IFZ<=0THENPRINT"Z (LENGTH OF INTEG) WAS="Z" I'VE RETURNED":RETURN
3720 IFE9<=0THENPRINT"ACCURACY (E9) WAS=";E9:E9=1E-04:PRINT" DEFAULT="E9
3730 W1=2:W2=4:W3=.375:W4=1.125:W5=1.5:W6=6:W7=24:W8=16:W9=7:W0=32:WW=12
3740 H=Z/(2*INT(Z/H/2)):WZ=45
3750 IFK9<X+W1*H THEN HH=H:H=(K9-X)/W1
3760 GOSUB3860:IF X >= K9 THEN K0=1
3770 X=X-H:FORK=1TON:Y9(K+N)=Y(K):Y(K)=Y1(K):NEXT:GOSUB3860:K7=0:FORK=1TON
3780   K2=ABS(Y9(K+N))*E9:K1=ABS(Y1(K)-Y9(K+N)):IFK1>K2 THEN 3830:REM BAD
3790   IF40*K1<K2 THEN K7=K7+1
3800 NEXTK:IFK0=1THEN3840:REM END
3810 WM=2:IFK7<N THEN WM=1
3820 X=X-H:H=WM*H:K8=X:FORK=1TON:Y(K)=Y9(K+N):Y9(K)=Y(K):NEXT:GOTO3750
3830 K=N:NEXTK:H=H/W1:X=K8:FORK=1TON:Y(K)=Y9(K):NEXT:K0=0:HH=0:GOTO3750
3840 X=X-H:IFHH<>0THENH=HH
3850 FORK=1TON:Y(K)=Y9(K+N):NEXT:RETURN
3860 REMARKABLE BUTCHER SUBROUTINE
3870 FORK=1TON:Y0(K)=Y(K):NEXT:K5=X:K6=H/W1:GOSUB3650:FORK=1TON:W1(K)=D(K)
3880   Y0(K)=Y(K)+K6*D(K):NEXT:X=K5+K6:K6=H/W2:GOSUB3650:FORK=1TON:W2(K)=D(K
)
3890   Y0(K)=Y(K)+K6*(W1(K)+D(K)):NEXT:GOSUB3650:X=K5+H:FORK=1TON:W3(K)=D(K)
3900   Y0(K)=Y(K)+H*(W1*D(K)-W2(K)):NEXT:GOSUB3650:X=K5+W5*H:FORK=1TON
3910   W4(K)=D(K):Y0(K)=Y(K)+H*(W3*W1(K)+W4*D(K)):NEXT:GOSUB3650:FORK=1TON
3920   W5(K)=D(K):Y1(K)=Y(K)+H*(W1(K)+W2*W3(K)+W4(K))/W6
3930   Y0(K)=Y(K)+H*(W2*W2(K)-W6*W1(K)+W7*(W3(K)-W4(K))+W8*D(K))/W9:NEXT
3940 X=K5+W1*H:GOSUB3650:FORK=1TON
3950   Y(K)=Y(K)+H*(W9*W1(K)+W0*(W3(K)+W5(K))+WW*W4(K)+W9*D(K))/WZ:NEXT:RETU
RN
3960 REM
3970 REM PARAMETER INPUT
3980 REM
3990 PRINT:PRINT
4000 PRINT "ENTER PARAMETRS DESCRIBING EXPERIMENTAL CONDITIONS"
4010 PRINT:INPUT"ENTER KM, PRESSURE DROP";KM,DPZ
4020 PRINT:INPUT"ENTER CB, VISCOSITY";CB,U
4030 PRINT:INPUT"ENTER INITIAL FLUX";YINIT
4040 PRINT:INPUT "ENTER TIME INTERVAL";Z
4050 PRINT
4060 RETURN
```

APPENDIX II

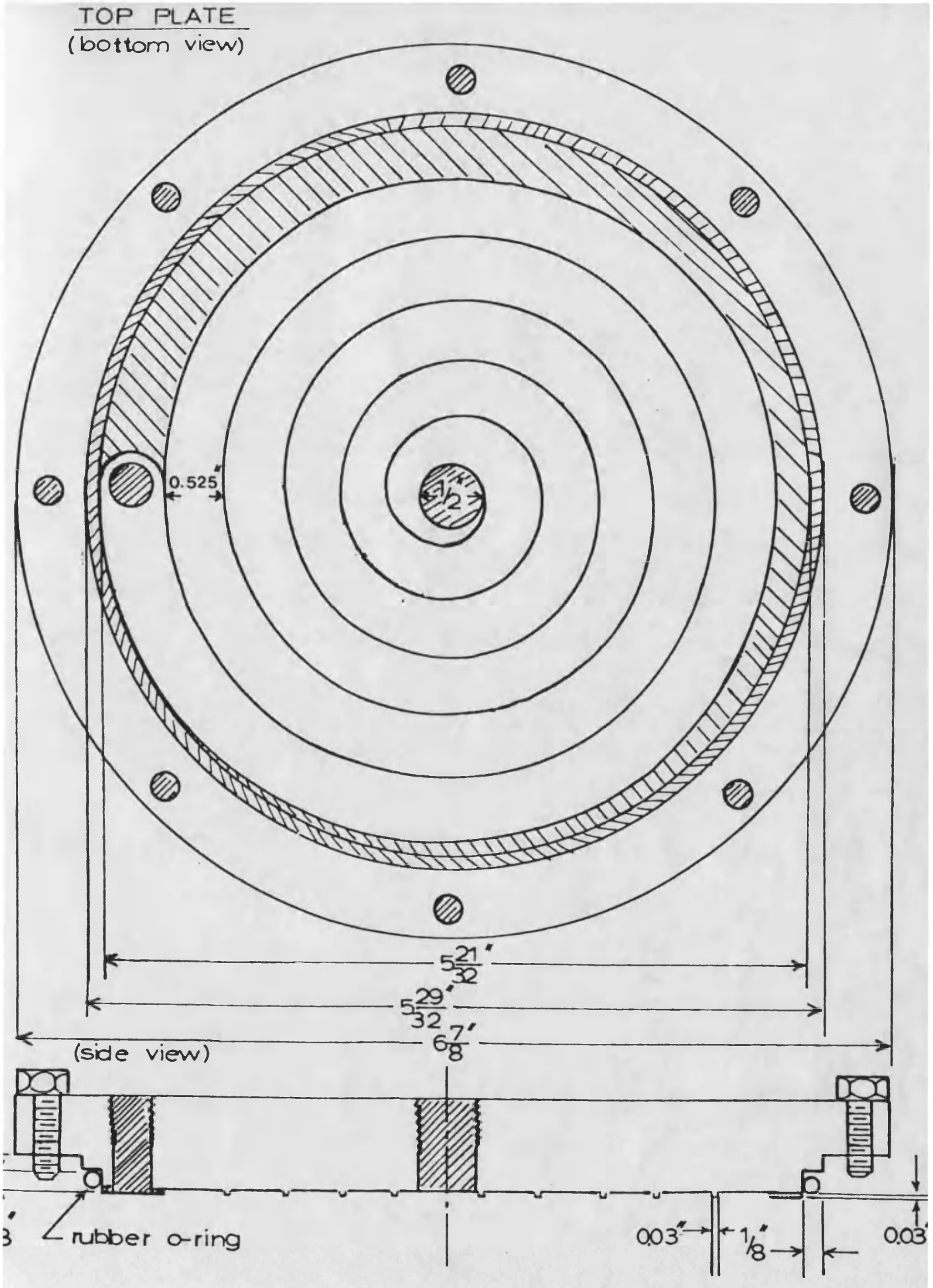
CHEMICALS

Ammonium sulphate ACS	Aldrich Chemical Co. Ltd.
α-Amylase	BDH Chemicals Ltd.
BSA No A-7906	Sigma Chemical Company.
Casein (technical)	Sigma Chemical Company.
Citric acid	BDH Chemicals Ltd.
L-Cysteine	BDH Chemicals Ltd.
EDTA	Sigma Chemical Company.
Ferrous sulphate	BDH Chemicals Ltd.
Hydrogen peroxide	BDH Chemicals Ltd.
Hypochlorid acid	BDH Chemicals Ltd.
Lactose MC 20	Boehringer Corporation Ltd.
0-Nitrophenyl-β-D-Galactopyranoside	Sigma Chemical Company.
Pepsin	BDH Chemicals Ltd.
di-Potassium hydrogen orthophosphate	Sigma Chemical Company.
Protamine sulphate (grade II)	Sigma Chemical Company.
Pyrophosphate tetrasodium	Sigma Chemical Company.
Sodium carbonate	BDH Chemicals Ltd.
Sodium hydroxide	BDH Chemicals Ltd.
Sodium perborate	BDH Chemicals Ltd.
Sodium phosphate	BDH Chemicals Ltd.
Terg-A-Zyme	Aldrich Chemical Co. Ltd.
Thiamine	Sigma Chemical Company.

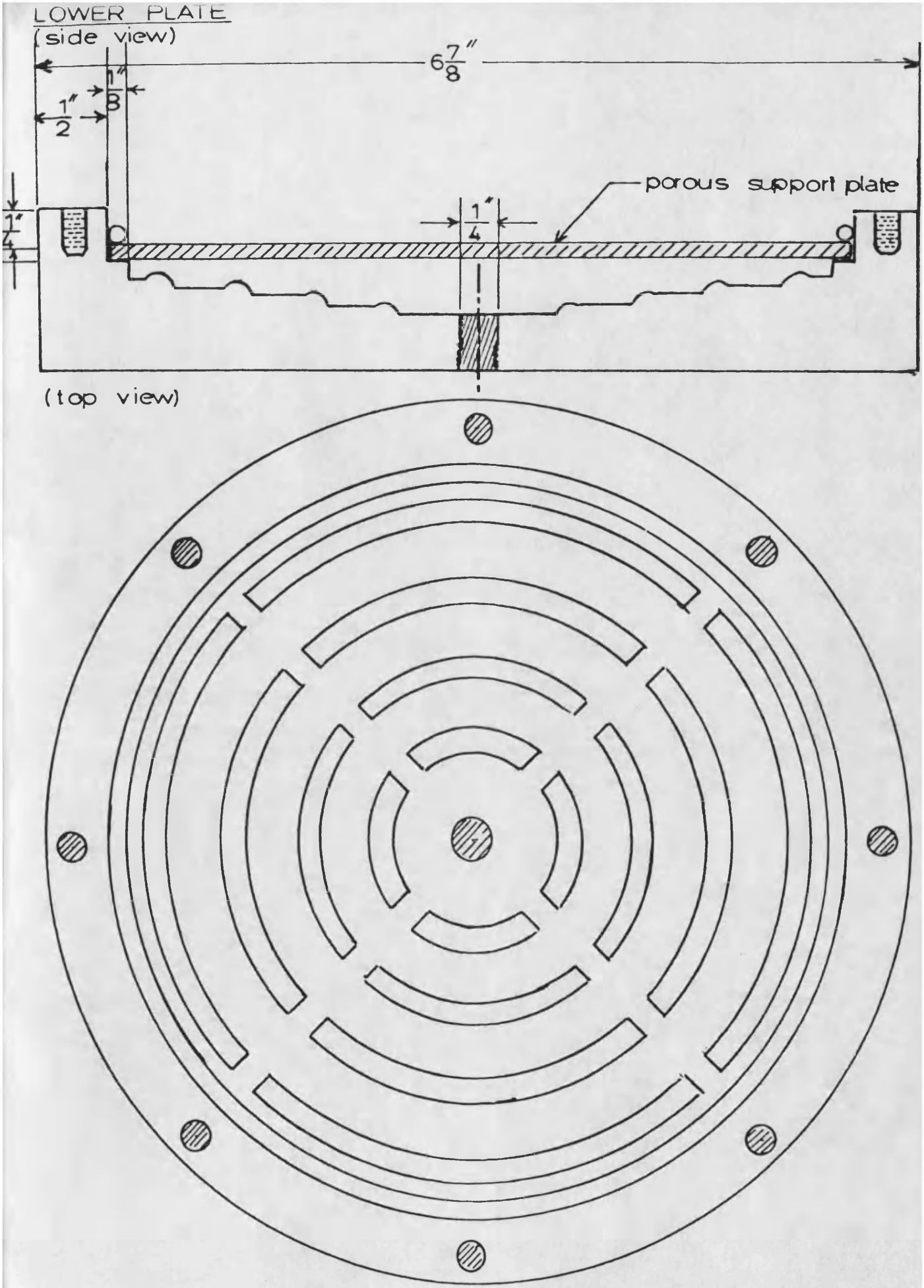
EQUIPMENT SPECIFICATIONS

Centrifugal pump Pump no: 12-E243302 X	Stuaet Turner Ltd.
Centrifuge Serial no: 235-TR	Micro Centaur MSE
Gauges, Schaffer diaphragm Pressure range: 0-60 PSI	Budenberg
Multiperistaltic pump Serial no: PWP7	Schuco Scientific Ltd.
Opto switches Stock no: 304-560	RS Components Ltd.
Personal computer Order code: 060010	BBC Model B
Prefilter, Ø 9cm Pore size: 20-25 μm	Whatman Ltd.
Prefilter housing, Ø 9cm Catalogue no: YY3009000	Millipore Corporation.
Printer	Epson LX-80
Rotameter Flow range: 0-2 ℓ/min	Rotameter Manufacturing Co.Ltd.
Solenoid valve Stock no: 349-715	RS Components Ltd.
Ultrasonic probe Serial no: SP-958	Ultrasonics Ltd.
UV-Spectrophotometer Serial no: 091026	CE 272 Linear Readout.
UV-Spectrophotometer Serial no: 08122	LKB 8300 UV-Cord II.

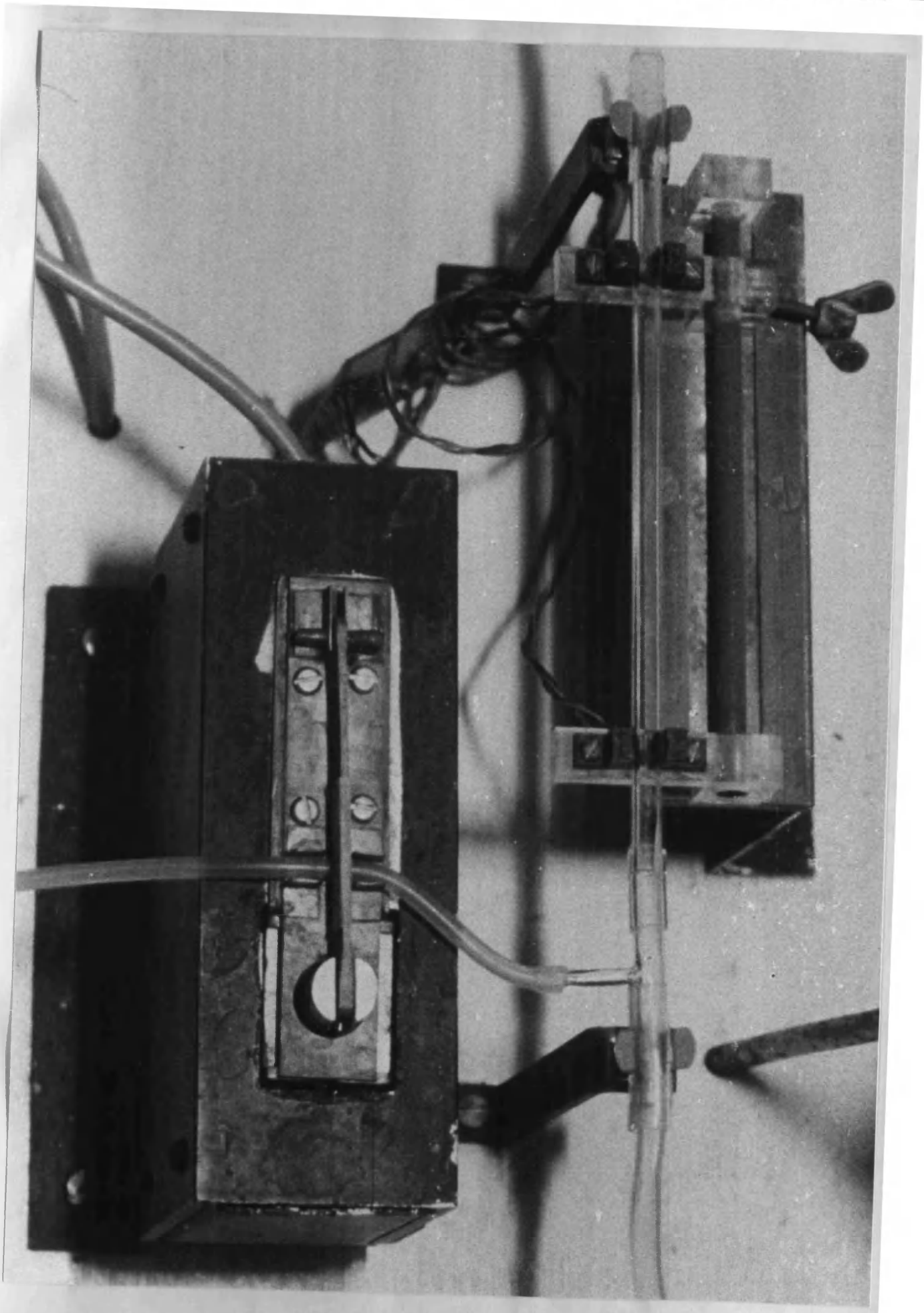
APPENDIX III



Detail of the thin-channel MF cell (top plate)



Detail of the thin-channel MF cell (bottom plate)



Flux measurement device (to scale)

NOMENCLATURE

Dimensions are given in terms of mass [M], length [L] and time [t].

C Solute concentration [M/L^3]

D Diffusion coefficient [L^2/t]

J Solvent flux [L/t]

K Membrane constant [L^2t/M]

K_R Reaction rate constant related to the growth of layer thickness

ΔP Transmembrane pressure drop [M/Lt^2]

P_g Permeability of the fouling layer [L^2]

Q Recycle flow rate [L/t]

R Membrane resistance [1/L]

Re Reynolds number

Sc Schmidt number

Sh Sherwood number.

b Channel depth [L]

d Channel diameter [L]

k_m Mass transfer coefficient for solute transport away from the membranes surface [L/t]

k_R Reaction rate constant related to the growth of resistance

ℓ Fouling layer thickness [L]

m Number of observations

n Order of reaction at which fouling takes place

t Time [t]

v Recycle flow velocity [L/t]

w Channel width [L]

GREEK LETTERS

$\bar{\epsilon}$ Porosity of the fouling layer [L^2]

ϵ Sum of least squares

μ Dynamic viscosity [M/L/t]

ν Kinematic viscosity [L^2/t]

π Osmotic pressure [M/L/t^2]

SUBSCRIPTS

A = Adsorbed

b = Bulk

bℓ = Boundary layer

g = Gel

m = Membrane

p = Pure water

s = at membrane surface

SUPERSCRIPT

* = at limiting conditions